The Organic Matter of the Soil: A Study of the Nitrogen Distribution in Different Soil Types

AUG 1918

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA

BY

CLARENCE AUSTIN MORROW, B.S., M.A.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

JUNE, 1918

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TO MY FATHER AND MOTHER

ACKNOWLEDGMENT.

This investigation was carried out under the direction of Doctor Ross Aiken Gortner. The author takes this opportunity to express his appreciation and gratitude for the help extended during the prosecution of this work, and for the unfailing kindness, thoughtful advice and encouragement which was given.

C. A. M.

University of Minnesota.
Division of Soils.
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I. INTRODUCTION: OUR PRESENT KNOWLEDGE OF THE ORGANIC MATTER OF THE SOIL.

A. Early Investigations.

It has been recognized from the time of the alchemists that manures are of fundamental importance for the growth of plants. The alchemists believed that by a process of transmutation water was converted into plant tissue. In an attempt to prove this an interesting experiment was conducted by van Helmont (1648). In a large earthen vessel he placed 200 pounds of dry earth, and planted in it a small willow tree weighing five pounds, and for five years he watered the plant either with rain or distilled water. At the end of that time he pulled up the willow and found that it weighed 169 pounds and three ounces. The dry soil remaining after the experiment was found to have lost only two ounces. He drew the apparently justifiable conclusion that 164 pounds of roots, bark, leaves, and branches had been produced by direct transmutation of water.

It is evident that it was essential to establish the composition of water and some of the components of the air before further work could have real value.

Not until the discovery of oxygen by Scheele (1777) and the proof of the composition of water by Cavendish (1784), as well as the work of de Saussure (1804) regarding the rôle played by carbon dioxide in plant and animal life, did we have any real knowledge concerning the sources of matter stored up in plants.

During the first quarter of the nineteenth century organic compounds were regarded as capable of being synthesized only in the living cells of plants or animals. This idea that organic compounds could be formed only through a special **vital force** was overthrown by the classic work of Wöhler (1828) when he prepared urea, a purely animal product*, by evaporating ammonium cyanate to dryness. This fact attracted the attention of chemists, and practically all work done from that time until 1840 was on some phase of organic chemistry. This overthrow of the belief in a vital force and the improved method of organic analysis by Berzelius (1808-18) paved the way for a more thorough understanding of the part taken by the organic matter in the soil, and consequently created a renewed interest in scientific investigations relating to agriculture.

Some important work had been accomplished previous to this time. The most valuable was that of de Saussure's (1804) "Recherches chimiques sur la végétation" which was the first systematic work showing the source of compounds stored up in the plant. He pointed out that the quantitative increase in the carbon, hydrogen, and oxygen, when plants were exposed to sunlight, was

*Fosse (1912) notes the presence of urea in certain plants.

due to the carbon dioxide of the air and water of the soil. He believed that the nitrogen of the soil was the chief source of the nitrogen found in plants. Unfortunately his conclusions were not accepted at that time, and it was not until about fifty years later, when other investigators had repeated his experiments, that his results were finally accepted by botanists and chemists.

One of the first investigators to see the relation between chemistry and agriculture was that of Sir Humphry Davy (1813), who published a book entitled, "Elements of Agricultural Chemistry." This treated of the composition of air, soil, manures, plants, and of the influence of heat and light upon the growth of plants.

Thaër (1809-10) contended that humus determined the fertility of the soil, that plants obtained their food mainly from humus, and that the carbon compounds of plants were produced from the organic carbon compounds of the soil. These ideas gave rise to his so-called humus theory, which was later shown to be inadequate. His writings, however, did much to stimulate later investigation.

The French investigator Boussingault verified much of the earlier work of de Saussure and secured many additional facts concerning the chemistry of growth. His predecessors had sought to solve the question as to whether plants assimilate the free or uncombined nitrogen of the atmosphere. Boussingault (1838, 1838a) improved the methods for the determination of the point in question, and showed that peas and clover could get their nitrogen from the air while wheat could not. Unfortunately, he did not make as much of this discovery as he might have done.

Boussingault was the first to have a chemical laboratory located on a farm and to make investigations along a practical line in connection with agriculture. **His was the first agricultural experiment station.**

The investigations of de Saussure, Boussingault, Davy, Thaër, and others paved the way for the work and writings of Liebig. He published (1840) "Organic Chemistry in its Application to Agriculture and Physiology," which was an important factor in attracting the attention of the public to agricultural problems. Many of his investigations and discoveries in the field of organic chemistry were applied directly to his interpretation of these problems.

He assailed the humus theory of Thaër and showed that humus could not be an adequate source of the plant's carbon. By applying the exact methods of chemistry to agriculture Liebig succeeded in establishing that plants derive the carbon of their tissues from the carbon dioxide of the air, and not from the carbon compounds that may be present in the soil. He came to regard the ammonia of the air as analogous with the carbon dioxide of the air, and preached the doctrine that plants were able to derive their nitrogenous food from the atmosphere. In the Farmer's Magazine, for instance, he writes:

> **If the soil be suitable, if it contains a sufficient quantity of alkalis, phosphates, and sulphates, nothing will be wanting. The plants will derive their ammonia from the atmosphere as they do carbonic acid. (Cited by Russell, 1912.)**

Although the work of Liebig was not conducted in connection with field experiments, it had a stimulating effect upon agricultural

investigation, and we are greatly indebted to him for summarizing previous work and pointing out valuable lines of future research.

In his book (1840) he states, that—

a rational system of agriculture cannot be formed without the application of scientific principles, for such a system must be based on an exact acquaintance with the means of nutrition of vegetables, and with the influence of soils, and actions of manures upon them. This knowledge we must seek from chemistry, which teaches the mode of investigating the composition and study of the character of the different substances from which plants derive their nourishment.

In one essential point, however, he fell into error. Lawes, the pioneer experimenter on agriculture in England, flatly denied the accuracy of Liebig's conclusions as regards nitrogen assimilation. The results of the investigations at Rothamsted as conducted by Lawes and Gilbert (1851) on the non-assimilation of atmospheric nitrogen by crops, were accepted as conclusive evidence upon this much discussed question.

The alkali soluble portion of the organic matter of the soil has formed for many years the subject of keen interest and discussion. This portion of the soil organic matter was called "humus" by the earlier writers, but this name has in more recent times been used by some American and most European investigators to designate the total organic matter of the soil. I have used the term throughout this paper in its original meaning. In early days the "humus" was regarded as being of very simple composition. De Saussure (1804) for instance, described it as a "brown combustible powder soluble in alkalies and ammonia compounds."

Klaproth* applied the name "ulmin" to dark colored amorphous bodies such as those obtained by Vauquelin (1797) from the bark of diseased elm trees. Sprengel (De Candolle** 1833, p. 280), who obtained similar bodies from soils applied to these the name "humic acid." Berzelius (1838) evidently had the general meaning of the term "humus" in mind when he used the term "humin" in describing certain dark colored constituents of vegetable mold. Following the use of the term "humin" as applied to what was considered to be a definite organic body, a number of other workers took up the study of similar substances, and a number of other terms more or less related, soon appeared. The name of Mulder (1849) is associated for the most part with the terms applied to humus-like substances which have appeared more or less in the literature from that time to the present. For instance, he says:

At present seven different organic substances are known to exist in the soil. They are crenic acid, apocrenic acid, geic acid, humic acid, and humin, ulmic acid and ulmin.

These bodies were divided by him into two groups, one consisting of crenic and apocrenic acids, and the other group embracing all the others. According to Mulder (1849) these seven organic bodies were intimately related, and five at least were five successive steps in the decay of organic matter in the soil. The first step in this decay he regarded as ulmic acid; this on further oxidation yielded humic acid; and this in its turn, on still further oxidation, geic acid. Continued oxidation produced apocrenic, and finally crenic acid.

*(De Candolle 1833, p. 279) states "Das Ulmin ist von Klaproth entdeckt worben."

**I have been unable to verify the original citation, which, according to Roper was Kastner's Archiv. Bd. 7, p. 163; Bd. 8, p. 145. Presumably one of these articles is that referred to by Russell (1912) entitled "Ueber Pflanzen-Naturlehre, Nürnberg, 1826.

A number of chemists have given the percentage composition of the supposed acids, but no two agree. Nothing is known in regard to their constitution. The lack of definite chemical characterization of these compounds is stated by Cameron and Bell (1905) as follows:

> The existence itself of these acids has never been satisfactorily demonstrated. * * * * No satisfactory description of the physical or chemical properties of these supposed acids, their salts, or characteristic derivatives, have been recorded.

Nearly every writer on soils from the time of Mulder to Hilgard (1906, p. 126) spoke of these acids with the same assurance as of oxalic or tartaric acids, or any other organic compound that has well known derivatives.

The early investigators, including Mulder, soon found that sugar, starch, carbohydrates generally, and even proteins, when treated with strong acids or alkalies, gave rise to dark colored compounds having the same general appearance and properties as the humus substances arising in the soil through decay. However, a conspicuous feature of the work on these humus substances is the discordant results for preparations bearing the same name and often from the same source.

Robertson, Irvine, and Dobson (1907) reached conclusions that although there were many strong resemblances between natural and artificial humus preparations, in regard to properties and composition, yet there are important differences in constitution. Recently Gortner (1916 c) has shown that in all probability the humin formed from carbohydrates is actually formed by a polymerization of furfural which is in turn formed from the carbohydrates by the action of the acid. Gortner and Blish (1915), Gortner (1916 c), and Gortner and Holm (1917) have likewise shown that the dark colored products originating in an acid hydrolysis of protein substances have their origin in the tryptophane nucleus. Obviously if carbohydrate humin originates from furfural, and protein humins originate in the tryptophane nucleus, mixtures of protein and carbohydrate would produce a great variety of physically similar but chemically different mixtures.

One of the important points at issue between the early investigators was whether humic acid and allied bodies contained nitrogen as a constituent. Mulder (1849, 1862) held that nitrogen was not a constituent of these substances, but was present as ammonia, that is the acids were present in the soil as ammonium salts, and in this connection he says:

> In good arable soil—that is, one in which the organic constituents are as far as possible decomposed—none of these substances contain nitrogen as a constituent element; all their nitrogen exists in the state of ammonia.

Detmer (1871) came to the opposite conclusion, claiming that nitrogen in humic acid as usually obtained, was present in organic combination (not, however, bound in the humic acid molecule). He obtained his humus by digesting with alkali. After precipitating with acid, he redissolved it in ammonia and precipitated the mineral constituents with phosphoric and oxalic acids and ammonium sulphide. After treating with potassium hydroxide and precipitating with hydrochloric acid, he obtained a preparation

containing 1.5 per cent nitrogen. No ammonia was evolved on making alkaline, but about 23 per cent of the total nitrogen was evolved on treatment with sodium hypobromite. Detmer believed, however, that humic acid did not contain nitrogen, but that the nitrogen found was present in some organic compound occurring as an impurity in his humic acid preparation. By a tedious process he was able to lower the nitrogen content of his humic acid to 0.179 per cent.

Ritthausen (1877) attributed the high nitrogen content of peat to the formation of complex, difficultly decomposable materials by absorption of ammonia and pointed to the low ammonia content as an indication of it, claiming it was not present as such after absorption. In an attempt to disprove Ritthausen's theory, Sivers (1880) found that he could expel only very small amounts of ammonia by heating with potassium hydroxide. He concluded that all the ammonia taken in remained as such, and did not go to form complex compounds. He maintained that most of the nitrogen was in the form of protein but presented no conclusive evidence. Grouven (1883) tried to show that the nitrogen of humus was due to the absorption of ammonia by humic acids, but found from various samples that only one-fiftieth of the total nitrogen was liberated on heating with milk of lime, and only one-twentieth when heated for two hours with potassium hydroxide.

It was found by Loges (1886) that the hydrochloric acid extract of the soil gave a precipitate with phosphotungstic acid, which is recognized as being a precipitating agent for certain nitrogenous compounds. Baumann (1887) found that certain black Russian soils rich in humus, containing but small traces of ammonia in the soil, gave a considerable amount of it on boiling the soil with dilute hydrochloric acid. From this he suggested the presence of amino and amide compounds in the soil. About the same time this subject was more thoroughly investigated by Berthelot and André (1886). They found that the nitrogenous matter was split up producing ammonia and soluble nitrogenous compounds, and that the hydrolysis goes further the greater the strength of the acid, the longer it is in contact with the soil and the higher the temperature. A soil containing 0.174 per cent of nitrogen was heated on a water bath for two hours with 7 per cent hydrochloric acid and 31.9 per cent of its nitrogen was dissolved. Of this soluble nitrogen 17.8 per cent was ammonia. Similar experiments were conducted using 3.4 per cent hydrochloric acid, and 0.7 per cent hydrochloric acid and distilled water.

Warington (1887) working with a sample of Rothamsted soil, which had been heavily manured, showed the presence of a small amount of amide nitrogen by using both hypobromite and nitrous acid. It seems highly probable from these experiments that at least a part of the nitrogen in the soil is present as amino compounds. Eggertz (1888) found that the nitrogen content of thirteen samples of humus varied from 2.59 to 6.43 per cent and states that the nitrogen was present in organic form and not as an ammonium salt. Sestini (1899) also showed the presence of amino

nitrogen by the action of nitrous acid. Dojarenko (1902) working with humus from seven Russian soils found appreciable quantities of amino nitrogen. Unlike previous investigators he determined the amount present quantitatively, and assumed all of the amino nitrogen was present as amino acids. He also made determinations of the ammonia by distillation with magnesium oxide, and obtained the amide nitrogen by hydrolysis with dilute hydrochloric acid and subsequent distillation of the ammonia formed with magnesium oxide.

B. The Humus Theory of Grandeau.

A tremendous impetus was given to the study of soils by the work of Grandeau, because he believed that the ammonia extract of soils contained the nutritive substances essential for the life of the plant and for the fertility of the soil. The theory that the humus extract was of such value had a great deal to do with retarding the development of the study of the organic matter of the soil.

The method of Grandeau (1872) is essentially the one in use at the present time in America for the determination of humus. He elaborated a method for the estimation of the "matière noire" of the soil by first leaching the soil with dilute acid in order to set the humus free from its combination with the alkaline earths, removing the excess of acid by washing with water, then moistening the soil with ammonia and allowing it to stand for a short time (three to four hours, cf. Grandeau 1877, p. 149), after which the humus solution was displaced by repeated washings with ammoniacal water. The dark brown solution so obtained was evaporated to dryness in platinum, weighed, ashed, and the amount of "matière noire" and of ash recorded. Grandeau regarded the humus ash as an integral part of the humus. He believed that the organic matter which dissolved was responsible for the fertility of the soil, apparently not so much because of the carbon and nitrogen content, as for the high percentage of phosphoric acid and potash in the humus ash.

The views of Grandeau were never generally adopted in Europe although often accepted by individual workers, but have been more generally accredited in America, due to the sponsorship of certain parts of Grandeau's humus theory by the late Professor Hilgard. The American investigators, e. g., Hilgard (1906), Ladd (1898), and Snyder (1895, 1897, and 1901), however, do not report the humus ash as an integral part of the humus but call only the volatile portion humus. They consider that the humus is, in part at least, combined in the soil with inorganic substances; these compounds are called "humates" and to their abundance and production has been ascribed an important part of the maintenance of soil fertility.

Hilgard (1906) regarded the humus of the soil as a definite soil product, formed from vegetable material in the soil under the influence of fungus and bacterial growths; this conversion being

most efficiently carried out in the presence of only a moderate amount of moisture, under the influence of a more or less rapid circulation of air and in the presence of calcium carbonate to neutralize any acids which may be formed. Under these conditions the vegetable substance is converted into black, neutral, insoluble humus compounds.

He believed that the nitrogen of plant debris which has become an integral part of the soil must first be converted by humifying bacteria and fungi into humus before the nitrogen can become available to the nitrifying bacteria and thus rendered available for the use of the higher plants. His views of the persistence of plant materials in soils are contained in the following statement:

> As a matter of course, the several organic compounds contained in plants may continue to exist in soils for some time, varying according to conditions of temperature and moisture. Thus dextrin, glucose, and even lecithin and nuclein have been reported to be found. The activity of the numerous fungus and bacterial ferments under favoring conditions, will of course, limit the continued existence of such compounds somewhat narrowly so that they can hardly be considered as active soil ingredients save in so far as they favor the development of bacterial flora.

Suzuki (1906-08 a) made a study of the formation of humus by treating oak leaves with a humus soil and various inorganic compounds and concluded that not only calcium carbonate but also magnesium carbonate promoted the decomposition of moist oak leaves by fungi, judging from the amounts of carbon dioxide evolved. He further states that the opinion of Hilgard corresponds closely to the natural conditions of humification. Further studies of Suzuki (1906-08 b) indicate that protein, starch, and pentosans contribute to the formation of the black matter of humus, but neither fat nor cellulose, and that protection from air is essential.

One of the latest additions to the idea of specific humification of plant materials in the soil is that of Trusov (1915). He inoculated various types of organic compounds with soil bacteria for various lengths of time and concludes that humus has its origin in lignin, albumen, starch, chlorophyll, and tannic substances; while cellulose, hemicelluloses, mono- and disaccharides, glucosides, organic acids including amino acids, and wax forming substances do not appear to have any part in its formation. He also finds that the organic nitrogenous compounds used as nutrients for the microorganisms may serve as an indirect source of humus.

Weir (1915) has recently questioned the idea that the soluble humus of the soil is an indication of the fertility of that soil and that the humus nitrogen plays an important rôle in the nutrition of plants. He removed 40 per cent of the nitrogen of the soil by extracting the humus with sodium hydroxide and then used the extracted soil for pot experiments. However, Gortner (1916 b) has shown that in all probability a very considerable portion of the humus nitrogen still remained in Weir's extracted soil, for he was able to extract 90.3 per cent of the original nitrogen content of the soil. This would indicate that nearly all of the soil nitrogen could be extracted with sodium hydroxide.

Snyder (1897) prepared artificial humus by mixing a subsoil with certain organic substances and allowing these to remain in a moist condition for one year. At the end of the year humus was

determined on the resulting mixture by extraction with ammonia, following a previous leaching with dilute acid, and the humus so found was regarded as having been formed in the soil during the preceding year. Unfortunately Snyder did not correct for ammonia soluble organic matter in the mixtures at the beginning of the experiment. The results of Fraps and Hamner (1910) and Gortner (1917) show that ammonia dissolves a considerable portion of material from unchanged organic compounds, so that the humus gain at the end of the experiment was in all probability actually a loss when compared with the amount of ammonia soluble materials at the beginning of the experiment. Fraps and Hamner (1910) as well as Gortner (1917) report a series of experiments after the general plan adopted by Snyder, with the exception that the ammonia soluble materials were determined both at the beginning and at the end of the experiments, and in each instance the ammonia soluble material was found to decrease.

The experiments of Gortner (1917) furnish no evidence that a specific "humification" of plant materials takes place in the soil giving rise to an increased amount of "humus." He says:

> On the contrary, all of the evidence is directly opposed to such a conclusion, and it appears altogether probable that the maximum amount of ammonia soluble material is present in a soil immediately after a green manuring crop has been plowed under and before the 'humifying' bacteria or fungi begin their work.

Fraps and Hamner (1910) showed that the humus extract of soil must contain substances from unchanged vegetable materials, while Gortner (1916 a) pointed out that the extract must contain substances from unchanged plant material, from bacteria and protozoa.

C. The Complexity of the Ammonia Soluble Material.

With the development of chemistry the idea has been gradually abandoned, that the ammonia soluble compounds can contain the whole of the organic matter which is responsible for the fertility of the soil. The work of the U. S. Bureau of Soils in the isolation of a large number of definite organic compounds from the soil, has been a distinct contribution along this line.

As has been suggested by Gortner (1916 a)—

> If one speculates on the nature of the soil organic matter, it becomes obvious that the variety of compounds which are present in a soil is limited only by those compounds which were present in the plants growing upon the soil, **plus** those compounds which compose the bodies of bacteria and protozoa, **plus** the compounds contained in the soil fungi, **plus** all the various compounds which may be formed from the above sources by decay, oxidation, and all the intricate chemical reactions which take place in converting dead organic material, either into living protoplasm on the one hand, or into water, carbon dioxide, and nitrogen on the other. Undoubtedly these organic compounds are not the product of 'humification' but are derived from unchanged plant material, from protozoa, or from bacteria.

A part, or all, of these compounds would be found in the "humus" extracted by Grandeau's method—

> Inasmuch as the 'humus' extract of soils is undoubtedly a mixture of organic compounds, many of which are colorless and in all probability are extracted from unchanged plant or animal materials, and inasmuch as the soil pigment present in this solution probably rarely exceeds 40 per cent of the 'humus', a determination of 'humus', as ordinarily carried out, appears to be wholly without scientific justification. (Gortner 1916 a.)

D. The Presence of Definite Organic Compounds in the Soil.

The following types of pure organic compounds have been isolated from the soil. In view of Gortner's statement above it is of interest to note that **all of these compounds are colorless,** and that nearly all of them were isolated from an **alkaline humus extract.**

1. Paraffin hydrocarbons.
   Hentriacontane, $C_{31}H_{64}$. Schreiner and Shorey (1910 a, 1911 a).
2. Alcohols
   Argosterol*, $C_{28}H_{44}O$. Schreiner and Shorey (1909, 1909 a)
   Phytosterol, $C_{28}H_{44}O$, H_2O. Schreiner and Shorey (1910 a, 1911 b)
3. Esters
   "Glycerides of fatty acids." Schreiner and Shorey (1910 a)
   "Resin esters." Schreiner and Shorey (1910 a)
4. Acids
   Oxalic acid, COOH–COOH. Shorey (1913)
   Succinic acid, COOH–CH_2–CH_2–COOH. Shorey (1913)
   Saccharic acid, COOH–$(CHOH)_4$–COOH. Shorey (1913)
   Acrylic acid, $CH_2 = CH$–COOH. Shorey (1913)
   α–Crotonic acid, CH_3–CH = CH–COOH. Walters and Wise (1916).
   α (1:11)–Monohydroxystearic acid, $CH_3(CH_2)_6CHOH(CH_2)_9COOH$. Schreiner and Shorey (1910 a, 1910 b)
   Dihydroxystearic acid, $CH_3(CH_2)_7CHOH$–$CHOH(CH_2)_7COOH$. Schreiner and Shorey (1908 a, 1909 a)
   Benzoic acid, C_6H_5COOH. Shorey (1914)
   Metaoxytoluic acid, OH–C_6H_3–CH_3–COOH. Shorey (1914)
   Agroceric acid, $C_{21}H_{42}O_3$. Schreiner and Shorey (1909 a)
   Paraffinic acid, $C_{24}H_{48}O_2$. Schreiner and Shorey (1910 a)
   Lignoceric acid, $C_{24}H_{48}O_2$. Schreiner and Shorey (1910 a)
   "Resin acids" (?). Schreiner and Shorey (1910 a)
5. Aldehydes
   Salicylic aldehyde, C_6H_4–OH–CHO. Shorey (1913)
   Vanillin, OH–$C_6H_3(OCH_3)$–CHO. m–methoxy–o–hydroxy benzaldehyde. Shorey (1914)
   Trithiobenzaldehyde, $(C_6H_5CSH)_3$. Shorey (1913)
6. Carbohydrates
   Mannitol, CH_2OH–$(CHOH)_4$–CH_2OH. Shorey (1913)
   Rhamnose, CH_3–$(CHOH)_4$–CHO, H_2O. Shorey (1913)
   Pentosan**, $C_5H_8O_4$. Schreiner and Shorey (1910 a), Shorey and Lathrop (1910)
7. Pyrimidine derivatives
   Cytosine, $C_4H_5N_3O$. 2–oxy, 6–amino pyrimidine. Schreiner and Shorey (1910 a, 1910 c)
8. Purine bases
   Xanthine, $C_5H_4N_4O_2$. 2, 6–dioxy purine. Schreiner and Shorey (1910 a, 1910 c)
   Hypoxanthine, $C_5H_4N_4O$. 6–oxy purine. Schreiner and Shorey (1910 a, 1910 c)
   Adenine, $C_5H_5N_5$. 6–amino purine. Shorey (1913)
   Guanine***, $C_5H_5N_5O$. 2–amino, 6–oxypurine. Lathrop (1912). Schreiner and Lathrop (1912).
   Tetracarbonimid****, $C_4H_4N_4O_4$. Shorey and Walters (1914)
9. Pyridine derivatives
   α–Picoline, γ–carboxylic acid, $C_7H_7NO_2$. Shorey (1906), Schreiner and Shorey (1908 b)

*The composition of the compound was determined by a single analysis, made with 0.1500 gram of the substance. It seems highly improbable that the composition could be obtained accurately from this amount of material.

**Pentose sugars have been separated as hydrolysis products of substances isolated from the soil (Schreiner and Shorey 1910 a).

***This compound was isolated from a steam heated soil.

**** This has been shown to be cyanuric acid (Wise and Walters 1917).

10. Amines
    - Trimethylamine, $(CH_3)_3N$. Shorey (1913)
    - Choline, $HON(CH_3)_3{-}CH_2{-}CH_2OH$. Trimethyl oxyethyl ammonium hydroxide. Shorey (1913)
    - Creatinine, $C_4H_7N_3O$. μ-imino (n) methyl α-keto tetrahydro glyoxalin. Shorey (1911), Schreiner, Shorey, Sullivan and Skinner (1911)
11. Organic phosphorus compounds
    - Nucleic acid. Shorey (1911 a, 1912, and 1913)
    - Lecithin, Aso (1904), Stoklasa (1911).
12. Amino acids
    - Arginine, $C_6H_{14}N_4O_2$. α-amino, δ-guanidine valerianic acid. Schreiner and Shorey (1910 a, 1910 d)
    - Histidine, $C_6H_9N_3O_2$. α-amino, β-imidazole propionic acid. Schreiner and Shorey (1910 a, 1910 d)
    - Lysine, $C_6H_{14}N_2O_2$. α ξ-di-amino, caproic acid. Shorey (1913)

Whether all of these compounds have actually been isolated is perhaps an open question, in view of the minute quantities which were obtained, insufficient in many cases for an exact chemical analysis as stated by Schreiner and Lathrop (1911):

> The amount of a substance obtained may be so small that extreme purification is out of the question, and therefore in such cases, where distinct crystalline form or characteristic tests are not available the identification becomes uncertain, as neither melting point nor analysis can be made.

E. The Origin of Organic Compounds in the Soil.

Chardet (1914) gives a discussion of the possible origin of certain organic nitrogenous compounds that have been isolated from the soil or might be expected to exist.

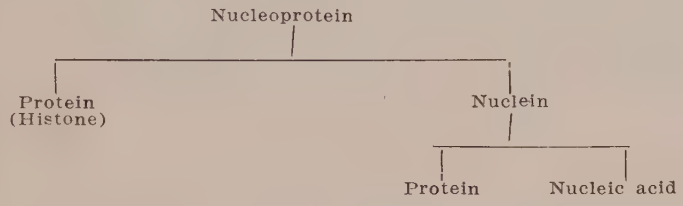
A very complete summary of our present knowledge of the organic matter of the soil is presented by Jodidi (1914) under these headings: I. Introduction; II. The sulphur compounds of the soil; III. The influence of certain factors on the quantity of nitrogen contained in the soil; IV. The nature of humus substances according to the older authors; V. The observations of later authors concerning the nature and behavior of humus to certain reagents; VI. Genetic relationship between the chemical compounds in the soil and those in plants and animals; VII. The nature of nitrogen compounds in the soil; VIII. The organic nitrogenous compounds of the soil; IX. Separation of the nitrogenous compounds in a sulfuric acid extract (i. e., hydrolysate) of the soil; X. Cleavage products of nucleoproteins; XI. Lecithin products in the soil; XII. Pyridine derivatives in the soil; XIII. Ammonification of amino acids and acid amides in the soil; XIV. The occurrence of hydrocarbons, alcohols, and aldehydes in the soil; and XV. The organic acids occurring in the soil.

Different investigators have succeeded in isolating from soils the following nitrogenous compounds which may be related to or derived from the proteins: tetracarbonimid, α-picoline γ-carboxylic acid, trimethylamine, nucleic acid, arginine, histidine, lysine, proteoses, and peptones. Potter and Snyder (1915 b) have shown that in some soils, at least, free amino acids and peptides occur but the amounts are very small.

Since there have been so many nitrogenous compounds isolat-

ed from the soil that are related to proteins, it is well to discuss some of the complex organic compounds such as nucleoproteins, nucleic acids, and lecithins that find their way to the soil through plant and animal remains. While the final decomposition products are undoubtedly simple compounds or elements such as carbon dioxide, methane, ammonia, nitrogen, and hydrogen, these products are reached by fairly definite and well defined methods of cleavage. The process may be a rapid one, a slow one, or one entirely arrested at certain stages, all depending on the factors present in the soil.

1. Nucleoprotein decomposition. The nucleoproteins are without doubt the most complex compounds that enter the soil. They are common constituents of plants, animals, bacteria, and molds, and hence occur wherever these live or die. The chemical changes through which these compounds go during decomposition may be rendered clear by the following scheme presented by Lilienfeld (1892):

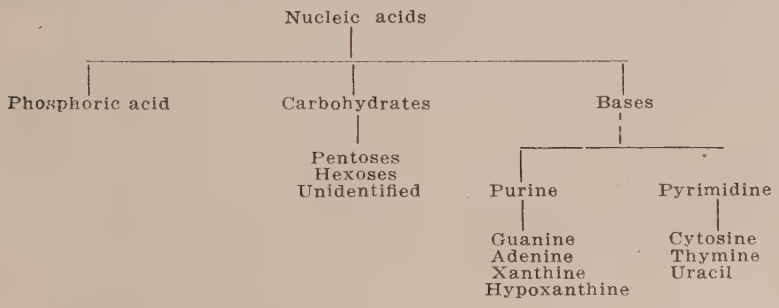


The products are then protein and nucleic acid, the latter of which has been isolated from the soil (Shorey 1911 a, 1912, and 1913).

2. Nucleic acid decomposition. Nucleic acids are constituents of all nuclei and on decomposition yield a variety of compounds composed of carbon, hydrogen, oxygen, nitrogen, and phosphorus. The acids occur in both plant and animal cells.

Jones (1914) states that all plant nucleic acids contain a pentose group, while on the other hand all animal nucleic acids yield levulinic acid, which is formed from a hexose group in their molecule.

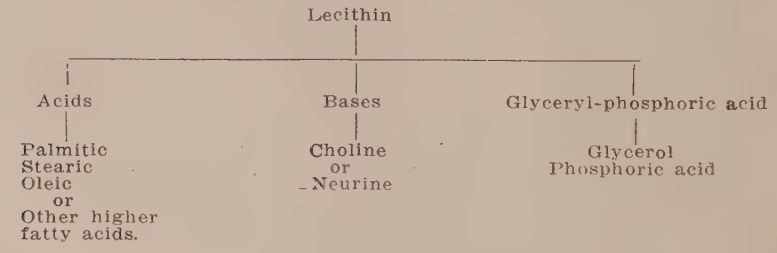
The hydrolysis products may be classified, according to Forbes and Keith (1914) as follows:



The purine and also the pyrimidine derivatives can be changed one into the other by chemical means. In a parallel manner much the same results can be obtained through the biochemical changes brought about by bacteria and enzymes. By chemical agents Kossel and Steudel (1903) transformed cytosine into uracil. In like manner Fischer (1882) changed guanine into xanthine, and Kossel (1886) changed adenine into hypoxanthine. It was shown by Schittenhelm and Schröter (1904) that putrefactive bacteria, especially those of the coli group, were able to convert guanine and adenine into xanthine and hypoxanthine and that bacteria also have the ability of breaking down nucleic acid itself. This change of nucleic acid is also accomplished by certain enzymes, the nucleases (cf. Jones 1914 and Euler 1912).

It will be clear from the above that the decomposition of nucleic acid may take place in many steps and that the intermediate as well as the final products may be transformed one into the other. This may be accomplished in the soil through the agency of microorganisms or enzymes. Schreiner and Lathrop (1912) working with steam heated soils found that from the heated samples less nucleic acid was obtained than from the unheated samples. On the other hand the decomposition products of nucleic acid were present in larger amounts in the heated soil, indicating that hydrolysis of nucleic acid has been accomplished in this manner. That nucleic acid decomposition does take place in the soil is evidenced by the isolation of certain of its decomposition products, e. g., cytosine, xanthine, hypoxanthine (Schreiner and Shorey 1910 a, 1910 c), adenine (Shorey 1913), and guanine (Lathrop 1912, Schreiner and Lathrop 1912).

3. Lecithin decomposition. A similar instance of a single substance decomposing into several substances is that of the lecithins. They are closely related to the fats in constitution and are possible primary constituents of all plant and animal cells. Lecithins are esters of glycerol with two molecules of higher fatty acids (palmitic, stearic, oleic acids or other unidentified saturated or unsaturated acids) with a molecule of phosphoric acid, which is at the same time combined with the base choline. Mathews (1915) states that in some cases choline can be replaced by neurine. There are a number of different lecithins which are characterized by the nature of the organic acid radicals present. The hydrolysis may be indicated as follows:



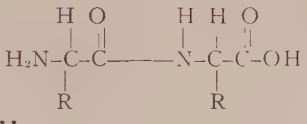
The base choline is also widely distributed in both plant and animal tissues as well as being a decomposition product of lecithins. It has been shown to exist in the soil. Choline yields neurine by bacterial decomposition, and both of these compounds break up into trimethylamine. This substance may also be added to the soil from other sources, both animal and vegetable. As noted above Stoklasa (1911) obtained evidences of lecithin in the soil and Aso (1904) reports small quantities of lecithin present in soils rich in organic matter. Choline has been isolated from soil (Shorey 1913). The trimethylamine reported by Shorey (1913) possibly had its origin in the lecithin molecule and it may be that the dihydroxy-stearic acid of Schreiner and Shorey (1908 a, 1909 a) and the monohydroxystearic acid (Schreiner and Shorey 1908 a, 1909 a) had the same origin. (For a discussion of the organic phosphorus of the soil see Gortner and Shaw 1917).

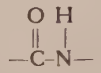
F. Bacterial Processes Which Influence the Form of Soil Nitrogen.

We know that the decomposition of protein substances can be brought about through bacterial activity or by the agency of enzymes widely distributed in the vegetable kingdom. We should expect any protein materials present in the soil to be subject to the action of the above agencies. Viewed in the light of the researches of Emil Fischer (1899-06) protein hydrolysis leads to disruption of the complex molecule and the formation of simple molecules, as represented in the following scheme—

Proteins——Proteoses——Peptones——Peptides——{ Di-amino acids / Mon-amino acids / Acid amides }

Fischer has shown that the amino acid combination in the protein molecule may be represented as follows:



The group  being known as the "peptid group." The nitrogen in this group is in the form of the imino (–NH) radical. Upon hydrolysis each "peptid group" takes up a molecule of water forming a free carboxyl (–COOH) group changing the imino group into an amino ($-NH_2$) group.

As the protein hydrolysis continues, the proportion of nitrogen in the amino form increases until it reaches a maximum at complete hydrolysis. It has been shown by Van Slyke (1910, 1911) that the amount of amino nitrogen formed is a measure of the hydrolysis of the protein substance. The amino acids derived from protein degradation may be acted upon by the bacteria in the soil and bring about chemical changes which depend largely on the character of the organisms present.

1. Deamination or reduction plays an important part in the formation of ammonium salts in the soil. The amino group is split off as ammonia and non-nitrogenous organic acids remain. It is not certain whether this process involves oxidation of the amino acid to the ketonic acid first, or whether the deamination is brought about by hydrolysis. If the hydroxy acids are first formed they are subsequently reduced so that the fatty acids are formed from the amino acids. This can be illustrated by the following examples:

Aspartic acid will give succinic acid, and this by loss of carbon dioxide gives propionic acid.

Tyrosine—> p-Hydroxy-phenyl-propionic acid—> p-Hydroxy-phenyl-acetic acid—> p-Cresol—> Phenol

This deamination or reduction is in all probability what is termed in soil chemistry **ammonification.**

2. Decarboxylation or amine formation involves the splitting off of carbon dioxide by the action of so-called carboxylase bacteria. This may happen either before or after deamination. Their formation is illustrated in the following reactions:

$CH_3-CH(NH_2)-COOH \longrightarrow CH_3-CH_2-NH_2+CO_2$
Alanine — Ethyl amine

$C_6H_4(OH)-CH_2-CH(NH_2)-COOH \longrightarrow C_6H_4(OH)-CH_2-CH_2-NH_2+CO_2$
Tyrosine — p-Hydroxy-phenyl-ethyl amine

$NH_2-(CH_2)_4-CH(NH_2)-COOH \longrightarrow NH_2-CH_2-CH_2-CH_2-CH_2-NH_2+CO_2$
Lysine — Cadaverine

$NH_2-C(NH)-NHCH_2-(CH_2)_2-CH(NH_2)-COOH \longrightarrow NH_2-C(NH)-NH-CH_2-(CH_2)_2-CH_2-NH_2+CO_2$
Arginine — Agmatine

Tryptophane gives rise to indole ethyl amine.

Mathews (1915) states—

If the splitting off of carbon dioxide occurs after the deamidization an amine cannot, of course, be formed, but the next lower carboxylic acid is produced by way of the aldehyde.

Thus from tyrosine there may first be formed p-hydroxy-phenyl-pyruvic acid, which may be reduced to p-hydroxy-phenyl-lactic acid, reabsorbed and reexcreted in the urine; or the p-hydroxy-phenyl-pyruvic acid may be split into p-hydroxy-phenyl acetaldehyde and carbon dioxide, and the former be oxidized into p-hydroxy-phenyl-acetic acid, which is excreted in the urine.

It is well to remember that any bacteria of the coli group will split off carbon dioxide from an amino acid. It is evident that mixed cultures of bacteria may be present in the soil and thus cause more than one type of splitting to take place.

3. Hydrolysis takes place with liberation of carbon dioxide. Ehrlich (1911) has shown that yeasts can convert amino acids into alcohols, liberating carbon dioxide and ammonia.

$C_6H_4(OH)-CH_2-CH(NH_2)-COOH+H_2O \longrightarrow C_6H_4(OH)-CH_2-CH_2OH+CO_2+NH_3$
Tyrosine — Tyrosol

4. Oxidation results with liberation of carbon dioxide and ammonia and the formation of a fatty acid containing one less carbon atom. A type reaction may be represented as follows:

$$R\text{–}CH_2\text{–}CH(NH_2)\text{–}COOH + O_2 \longrightarrow R\text{–}CH_2\text{–}COOH + CO_2 + NH_3$$

That oxidation is a factor in the organic matter of the soil is self-evident from the fact that carbon dioxide is constantly present in the soil atmosphere in excess of the amount present in the air, thus representing degradation of the organic matter to carbon dioxide and water, and also from the fact that ammonia is transformed into nitrates, a process known in soil chemistry as **nitrification,** a reaction which is carried out in the laboratory by the most violent chemical oxidation, **e. g.,** chromic acid. A further step in this oxidation carries the nitrates through **denitrification** which results in the liberation of free nitrogen.

In carrying to completion these processes on protein material one can easily postulate an almost unlimited number of organic compounds, which are theoretically (and in all probability) possible. Very recently Robbins (1916) has produced some evidence that the existence of certain of the organic compounds in the soil is limited somewhat narrowly by specific bacteria, which either utilize the nitrogen or the carbon of the compound as a source of energy. Thus pyridine is destroyed by a specific bacterium which is able to utilize the nitrogen, and the carbon of cumarin and vanillin is likewise a source of carbon for other specific bacteria.

G. Nitrogen Distribution in the Soil

The chemistry of soil nitrogen may to a large extent be considered as being the chemistry of protein undergoing hydrolysis. The isolation of a number of amino acids indicates that proteins are decomposed in the soil in much the same way as in acid hydrolysis or animal digestion. Just how far the cleavages have already gone in the soil previous to acid hydrolysis remains a matter of much work before definite conclusions can be drawn.

Walters (1915) has reported the presence of certain decomposition products in the soil, presumably proteoses and peptones, resulting from either a partial hydrolysis of proteins or by the synthetic action of microorganisms. It has been recorded by Hoppe-Seyler (1909, p. 413) that intermediate protein decomposition products may result from the action of water at high temperature, by mineral acids, alkalies, oxidizing agents, enzymes and microorganisms. There is little reason to suppose that the action of microorganisms is other than that of the enzymes which they produce. Effront (1914) states that under the influence of the various trypsins secreted by putrefactive bacteria, the protein molecule is split into proteoses, peptones and amino acids. The proteoses and peptones represent stages of decomposition between that of true proteins and amino acids. Walters concludes—

> that proteins undergo hydrolytic decomposition in the soil in much the same way as in digestion by enzymes, acids, or alkalies, in the laboratory.

In an extensive examination of the nitrogen compounds of processed fertilizers, Lathrop (1914) has reported the presence of certain protein-like substances similar to those described above.

In his studies on the chemical nature of the organic nitrogen in the soil, Jodidi (1911) thought water would be preferable to either acids or alkalies for the purpose of extraction, since it would not be so liable to alter the organic nitrogenous materials. He found that the direct extraction of a soil by boiling with water for ten hours removed only 2.92 per cent, and for twenty-four hours the highest amount removed from any soil was 9.96 per cent of the total soil nitrogen. Shmook (1914), however, reports 19.10 per cent of the total nitrogen of a Laterite soil of Russia to be water soluble.

The literature has been very thoroughly summarized by Potter and Snyder (1914) in regard to the determination of ammonia in soils. Both their work and that of Jodidi (1909) indicates that the amount of ammonia is small. Kelley and Thompson (1914) in a study of some Hawaiian soils reached the conclusion that ammonia and nitrate nitrogen constitute but a small percentage of the total nitrogen, and that the nitrogen is very largely in organic combination.

It is known that only a small part of the soil nitrogen is dissolved by dilute acids, yet it has been shown by Kelley and Thompson (1914) that 1 per cent hydrochloric acid dissolves some organic nitrogen, for in every instance the soils contained only about half as much ammonia nitrogen as was extracted by the acid.

In the soil studies of Potter and Snyder (1915 a) they find that the nitrogen extracted by 1 per cent hydrochloric acid varied from about 1.2 to 2.3 per cent of the total nitrogen, except in the case of the peat it was only 0.67 per cent. This is contrary to the findings of Gortner (1916 a). Working with eight mineral soils he finds a maximum of 4.18 per cent of the total nitrogen soluble in 1 per cent hydrochloric acid with an average of 3.17 per cent. In three peats he finds a maximum of 7.50 per cent with an average of 3.78 per cent, and in five samples of unchanged vegetable materials (oat straw, alfalfa hay, oak leaves, sweet fern leaves, and grass from a peat bog) he finds a maximum of 34.58 per cent with an average of 20.10 per cent. These findings would seem to indicate that in the transformation of vegetable materials into the true organic matter of the soil there is a fall in the proportion of the total nitrogen soluble in very dilute acids.

Shorey (1905) published results of his investigations which gave the first definite knowledge of the individual amino acids formed in the decomposition of soil organic matter. He worked on a Hawaiian soil with a view to classifying the decomposition products of the nitrogenous substances in the soil. The method applied was that proposed by Osborne and Harris (1903) for classifying the decomposition products of proteins resulting from acid hydrolysis. The method is a modification of that proposed by Hausmann (1899) and is in short as follows: After hydrolysis the excess of the

mineral acid is removed by evaporation, and the nitrogen present as ammonia determined by distilling with an excess of magnesium oxide; after separating the magnesia precipitate from the remaining solution by filtration, the nitrogen was determined in the precipitate by the Kjeldahl method, the di-amino nitrogen in the filtrate was precipitated by phosphotungstic acid and determined by the method of Kjeldahl and the mon-amino nitrogen determined by difference.

He obtained in the acid solution 84.5 per cent of the total nitrogen in the soil, 52.3 per cent of which was found in the magnesia precipitate. This result is in striking contrast to those obtained by Osborne and Harris (1903) working on pure proteins, where they found that the nitrogen contained in the magnesia precipitate does not usually exceed 4 per cent of the total nitrogen and in most cases is very much less. The amount of nitrogen insoluble in the 12 per cent acids used in the digestion may be designated as "humin." The nitrogen in the magnesia precipitate has been designated by most investigators "humin" nitrogen. The total "humin" nitrogen in the soil is then represented by the nitrogen in the magnesia precipitate plus that retained by the soil. On recalculation of his data it was found that the insoluble humin in the soil after hydrolysis amounted to 15.3 per cent of the total nitrogen, making a total humin nitrogen content of 59.1 per cent. This very high result of total humin nitrogen was undoubtedly due to the soil being hydrolyzed only seven hours with a relatively low concentration of hydrochloric acid and the insoluble residue boiled the same length of time with sulfuric acid. Complete decomposition of the proteins probably did not take place in the dilute acids used in the short time that they were heated. As a result partially hydrolyzed residues may have been precipitated by the magnesium oxide, which would account for the high results.

Shorey (1906) concluded that even though we might know much concerning the constitution of the compounds comprising the various groups isolated from protein by this method of analysis, we know nothing concerning their composition when isolated from soil, inasmuch as we are not dealing with a pure protein (cf. also Gortner 1913, 1914, 1916 c).

The work of Suzuki (1906-08 c) gives us further knowledge of the individual amino compounds formed in the decomposition of soil organic matter. He worked with three samples of humic acid, one obtained from Merck (origin unknown to Suzuki), one prepared from an unmanured soil, and one from a compost heap. After boiling each preparation for ten hours with strong hydrochloric acid, the undecomposed residue was filtered off, washed, and the residue extracted twice in this manner with strong hydrochloric acid. He determined the amounts dissolved as amide, di-amino, and mon-amino acid nitrogen. From 65 to 75 per cent of the total nitrogen was dissolved by the hydrochloric acid and in the extract 41 to 62 per cent of the nitrogen was not precipitated by phosphotungstic acid. A sample of humic acid was twice extracted, with

concentrated acid and the residue analyzed. His results calculated on the ash free basis showed the residue to contain 64.11 per cent carbon, 3.35 per cent hydrogen, and 0.80 per cent nitrogen. The residue becomes lower in nitrogen*, hydrogen, and ash but richer in carbon as the hydrolysis is continued.

Detmer (1871) pointed out that similar results were true in peat beds where the deposits remained undisturbed for years. He found that there is an increasing carbon and nitrogen content of the humus for varying depths. This is shown by the following table:

	Carbon	Hydrogen	Oxygen	Nitrogen
Brown peat, near the surface...	57.75	5.43	36.02	0.80
Dark peat, 7 feet.....	62.02	5.21	30.67	2.10
Black peat, 14 feet.....	64.07	5.01	26.87	4.05

Likewise Gortner (1917) observed—

that there is a much greater wastage of carbon than nitrogen. Hilgard (1906) calls attention to the increased nitrogen content of the humus over that of the original vegetable materials. If we take the average carbon content of proteins as 51.15 per cent (average of 30 analyses given by Mathews 1915) a C:N ratio of 3.06 found in soil A-1916 would give a nitrogen content of 16.71 per cent, which approaches very nearly to the average nitrogen content of these 30 proteins, i. e., 17.66 per cent. It is evident that the materials remaining in the soils are rapidly increasing in nitrogen content.

Suzuki (1906-08 c) made further studies on a 500 gram sample of the humic acid obtained from Merck. It was hydrolyzed with concentrated acid and the solution obtained subjected to esterification and fractional distillation according to the method of Fischer (1901). He obtained:

Alanine	2.39 gm.
Leucine	2.16 gm.
Alanine+aminovalerianic acid	0.11 gm.
Aminovalerianic acid	0.57 gm.
Proline (copper salt of active proline)	0.67 gm.
(copper salt of inactive proline) (?)	0.50 gm.
Aspartic acid	0.06 gm.
Impure aspartic acid (?)	3.16 gm.
Glutaminic acid	present
Tyrosine	trace
Histidine	trace
Ammonia	1.90 gm.
Copper salts of unknown acids	30.30 gm.

As these compounds are typical protein decomposition products, his work proves that the humic acid examined by him was either of a protein nature, a mixture of protein decomposition products, or probably both together with some as yet unknown compounds. Unfortunately, the origin of the acid was unknown to Suzuki, but he states that it was probably prepared from peat.

From a study on Michigan peat soils Jodidi (1909) has concluded that the bulk of the organic nitrogen is made up of acid amides, di-amino acids, and mon-amino acids. He used slightly modified methods. The ammonia was determined as above by distillation with magnesium oxide. The residue from distillation with magnesia was dissolved in dilute sulfuric acid and the

*He stated that although the nitrogen content decreases, it is very difficult to entirely remove all of the nitrogen.

di-amino acids precipitated by phosphotungstic acid. The nitrogen in the precipitate of di-amino acids was determined by the method of Kjeldahl. The filtrate from the di-amino acids containing the mon-amino acids was oxidized by the Kjeldahl method and the nitrogen determined. In some cases he secured the mon-amino nitrogen by difference, stating that it was difficult to get a direct determination of the mon-amino nitrogen by the Kjeldahl method.

He states that—

this percentage was usually higher than the one directly found by kjeldahlizing the filtrate from the phosphotungistic acid precipitate.

In one experiment the percentage of mon-amino nitrogen by direct determination was 62.83 per cent, while by difference the result was 67.22, and in another case the results were 64.25 and 65.06 respectively.

It will be noted that this is a departure from the method used by Shorey (1905) in that here the nitrogen is separated into **three** fractions instead of the usual four. The nitrogen in the magnesia precipitate was distributed with the di-amino and mon-amino acid nitrogen. This method of nitrogen distribution will be classed as "Jodidi numbers" (in contrast to the Hausmann numbers) in the subsequent portion of the paper.

Van Slyke's (1910) nitrous acid method was first applied by Robinson (1911) to a study of peat soil, in order to determine the amount of amino nitrogen present. The ammonia nitrogen was removed by previous distillation with magnesium oxide. The only value of Robinson's work seems to be in the fact that his figures for total and amino nitrogen increase to a maximum with increasing time of hydrolysis, in much the same manner that proteins react; thus indicating that the amino groups were not existing free in the peat but in some form of combination which did not react with nitrous acid. For example, after one hour's hydrolysis the total nitrogen of the soil in solution amounted to 29.86 per cent, while the amino nitrogen was 4.62 per cent or a ratio exceeding 6:1. After forty-two hours' hydrolysis the nitrogen of the soil in solution was 51.54 per cent of the total nitrogen and the amino nitrogen was 25.07 or a ratio only slightly exceeding 2:1. This ratio increases again with further hydrolysis so that at the end of 138 hours the ratio is almost 3:1. However, the amount of nitrogen in solution was so small that the experimental error of measuring total and amino nitrogen must have been quite large.

More recently Jodidi (1911) made a study of some Iowa soils using a modification of the Osborne and Harris (1903) method. The nitrogen removed from the solution by the magnesium oxide was ignored by the author.* This contained a part of the so-called "humin" nitrogen. Subtracting the sum of the ammonia** and di-amino nitrogen from 100 he found the per cent of the nitrogen in

*Experimental data presented later in this paper will show that this fraction may exceed 9 per cent of the total nitrogen.

**He distinguishes the ammonia nitrogen originally present in the soil as such, from that produced by acid hydrolysis.

solution as mon-amino nitrogen. It will be readily seen this conclusion is incorrect. The mon-amino acid nitrogen as determined represents the sum of the humin and mon-amino nitrogen. It is very unfortunate that this mistake should have been made since this gives us only the actual ammonia and di-amino acid nitrogen for use in comparison with other investigations of the organic soil nitrogen as distributed by acid hydrolysis. Kelley (1914) following details as outlined by Jodidi (1911) has made the same error and the criticisms above apply with equal force to his data.

It is also extremely unfortunate that investigators should in any case rely upon figures obtained "by difference" for any one of their fractions. It is sometimes permissible to use figures obtained in this manner, for example, in case a determination has been lost and lack of time or other consideration prevents a repetition; but to constantly use figures obtained in this manner is unscientific, especially, since by this method we have no means of determining how great the experimental error of the method may have been.

Jodidi (1911) called attention to the fact that in the case of protein substances the distillation of the hydrolyzed protein with magnesium oxide gives pure ammonia. This, however, may not hold true for the hydrolyzed portion of soils, since some protein substances through decay yield organic bases. It has been shown by Bocklisch (1885) that dimethyl amine is formed through putrefaction of fish, and trimethylamine has been produced by the putrefaction of wheat flour and fish. The bases putrescine and cadaverine result from the decay of organic substances under certain conditions. It is possible for certain di-amino acids to become transformed into di-amines, as for example, arginine can be decomposed into urea and ornithine through bacterial activity. These processes can be expressed by the following equations:

$$\underset{\text{Arginine}}{NH_2\text{-}C(NH)\text{-}NH\text{-}CH_2\text{-}(CH_2)_2\text{-}CH(NH_2)\text{-}COOH} + H_2O \longrightarrow$$

$$NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH + \underset{\text{Urea}}{\overset{\overset{NH_2}{|}}{C}=O}$$

$$\underset{\text{Ornithine}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH} \longrightarrow$$

$$CO_2 + \underset{\text{Putrescine}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2}$$

Jodidi found that the ammonia obtained by distilling the evaporated extract of the soil with magnesium oxide was actually pure ammonia, thereby establishing the absence of any volatile organic bases; but that the phosphotungstic acid precipitate and the filtrate from that precipitate did not represent di-amino and mon-amino acids only.

In order to find out how much of the di-amino and mon-amino nitrogen actually belonged to di-amino and mon-amino acids, the solutions were subjected to analysis by the formaldehyde-titration

method of Schiff (1900, 1901, 1902) as modified by Sörensen (1908), Henriques (1909), and Henriques and Sörensen (1910).

In a comparison of the amount of di-amino* acid nitrogen, calculated as if histidine, arginine, and lysine were present in about equal amounts, he finds that in plot E, 101.8 per cent; plot Q, 84.8 per cent; and plot U, 93.9 per cent of the nitrogen in the phosphotungstate fraction was actually present as di-amino acids.

However, he obtains widely divergent results for mon-amino acid nitrogen; in plot E, 91.64 per cent; plot Q, 52.63 per cent; plot U, 40.12 per cent; plot H, 88.31 per cent; and plot J, 92.11 per cent of the total nitrogen in the "filtrate from the bases" was actually present as mon-amino acid nitrogen whereas all should have been present in this form if dealing with pure proteins only. He, therefore, concludes that the di-amino and mon-amino acids, or in other words, **the bases and filtrate from the bases by hydrolyzing soils, contain other products than are formed by hydrolysis of pure proteins.**

In a series of fertilized soils studied by Lathrop and Brown (1911) they find that almost 98 per cent of the nitrogen in the soil is of organic nature. The ammonia and nitrate nitrogen constitute the remainder. Employing the same method to the distribution of the soil organic nitrogen as Shorey (1905), they boiled 100 grams of soil with 500 cc. of hydrochloric acid (sp. gr. 1.115) for three hours, and used the filtrate after making to definite volume for the analyses. The figures given for ammonia nitrogen represent the actual amount of nitrogen as ammonia obtained by hydrolysis and does not include the ammonia nitrogen already present in the soil. They find that the plots which have received organic fertilizers give the largest amount of ammonia on hydrolysis, the amount being highest in the plot which has received manure alone and lowest in the check plot.

Of the five soils studied, four contained over 25 per cent of the "humin" nitrogen soluble in acid, while the other only showed about half as much. Since the nitrogen of the soil not soluble in acid may be considered "humin" nitrogen, the total amount in this form in the above four soils was over 53 per cent, while in the other soil, which received dried blood, it amounted to only 43 per cent.

However, the fractions which they determined have actually very little significance in a discussion of protein hydrolysis products, inasmuch as a three-hour hydrolysis is far too short a time to completely decompose the protein molecule. This explains their high figures for humin nitrogen and low ones for mon-amino acid nitrogen. The di-amino and mon-amino acid nitrogen differ rather widely but there seems to be no agreement between the form of nitrogen and the plot treatment.

*The exact interpretation of his data is difficult to understand.

In conclusion they say—

these five samples of soil are really the same soil under long continued treatment of different kinds. It is not improbable that work on widely different soils will show even much greater variations than those here noted. The work shows, however, that even in such cases there is a difference in the nitrogenous compounds in the soil, and that different decompositions of the nitrogenous matter has taken place and probably will continue to take place, under the different conditions imposed upon the soils in the field.

A very interesting study has been made by Shmook (1914) of the nitrogen distribution in four Russian soils, one of the Podzol type, two of the Chernozem type, and one of the Laterite type by applying the method of Hausmann (1899). The water extract from 100 grams of the Podzol soil showed a very high content of soluble nitrogenous compounds. This amounted to 0.0452 gram of nitrogen which constituted 19.10 per cent of the total soil nitrogen. This was distributed as follows: amide nitrogen 0.0034 gram, di-amino nitrogen traces, and mon-amino nitrogen 0.0408 gram. These results were deducted from the analyses of the hydrolyzed soil. Thirty gram samples of soil were hydrolyzed with 120 cc. of hydrochloric acid (sp. gr. 1.12) for 8 hours and the analysis carried out as directed.

He finds, that the Chernozem and Podzol soils show a similarity in the distribution of amide nitrogen, and that of the amino acid nitrogen, but that the nitrogen distribution in the Laterite soil is entirely different from the other types. He concludes that the amount of protein in the soil is not in direct relation to the amount of organic matter, and that the nitrogen insoluble in hydrochloric acid occurs in unknown form and composes only 1.50 to 1.90 per cent of the organic matter of the Chernozem and Podzol soils, but 13.70 per cent of the total organic matter of the Laterite soil, after subtraction of the protein nitrogen belonging to this insoluble portion. He suggests that these results would indicate that the organic nitrogen existed in the soil in large part as protein material in the Chernozem and Podzol soils, but that a considerable portion was of a non-protein origin in the Laterite soil, since the amount of this insoluble "melanin" in pure proteins amounts to from 0.60 to 1.80 per cent of the total protein nitrogen.*

Potter and Snyder (1915 a) made a study of some Iowa soils using Van Slyke's (1911) method of protein analysis. Their soils were the same type but had received different fertilizer treatment. At the same time they also made a study of peat soil. The soils were in all cases first extracted with 1 per cent hydrochloric acid "in order to render the humus more soluble." They were then hydrolyzed by boiling one part of the soil with two parts of 22 per cent hydrochloric acid for forty-eight hours. They also prepared a 1 per cent sodium hydroxide extract of the acid leached soils, and after precipitation with sulfuric and acetic acids the resulting humic acid precipitate was subjected to the above method of analysis.

The authors conclude: (1) that the humin nitrogen as determined by the Van Slyke method on soils extracted by dilute alkali

*Actually in some cases these results are much lower, and in others decidedly higher, **e. g., Van Slyke (1911) finds gelatin contains 0.07 per cent and** fibrin 3.17 per cent.

is very high when compared to the amounts in proteins; (2) that no typical class of organic compounds is extracted from the soil by dilute alkali; (3) that the amounts of amino acid and peptid nitrogen in the soil are found to be very small compared to the amounts of amino acids formed by acid hydrolysis; (4) (and this is the most important for our purpose) that

nothing very significant can be deduced from the variations in the different soils.

or in other words, **the organic matter in the same soil type under different fertilizer treatment, is essentially the same, and as I shall show later in the experimental part of this paper, the organic matter (as distributed by Van Slyke's method) in different soil types is essentially the same.**

Lathrop (1916) recently made a study of protein decomposition in the soil. He added a high grade nitrogenous fertilizer to the soil and allowed decomposition to proceed at laboratory temperature, and at different periods took samples and subjected them to Van Slyke's method of protein analysis in order to determine how the different fractions were affected by bacteria and other agencies present in the soil.

From his work he concludes that the analysis obtained by the Van Slyke method indicates that there is a formation of protein taking place in the soil in the course of the decomposition of the protein materials, and that apparently the new protein is somewhat resistant to decomposition. He states, that—

this is indicated in (1) the unequal loss of mon-amino acids and hydrolyzable nitrogen from the soil during the early stages, (2) by an increase in amide nitrogen during the early stages, (3) by an increase in histidine nitrogen during the early stages, (4) by an increase in the arginine nitrogen during the later stages, and (5) by an increase in lysine nitrogen during the later stages.

This view that the protein nitrogen in the soil was largely contained in the bodies of bacteria and protozoa had been previously advanced by Shmook (1914).

It was stated by Loew and Aso (1906-08) that under favorable condition of growth protein material is excreted by yeast and bacteria, and that soluble materials can pass through the cytoplasm to the outside on death of the cell. They also state that the amount of nitrogenous substances partly consisting of peptones excreted by dead cells, is by no means inconsiderable.

H. A Summary of the Nature of the Organic Matter of the Soil in the Light of Our Present Knowledge.

It has been pointed out, that the organic matter of the soil was at first considered to be a very simple thing, that the alkali extract contained the essential plant nutrients, and that the process of "humification" was the necessary step through which the organic matter of the oil must pass in order to be converted into food materials for plant life; but as the knowledge of chemistry developed it became evident that the problem was more complex. A definite knowledge of the forms of organic matter in the soil can only be secured when we have a thorough understanding of

the chemical products formed by the action of the bacteria, protozoa, and fungi on each other, and on the organic matter, both animal and plant, that finds its way to the soil. The present method is to study the soil with its complex mixture of organic and inorganic compounds, and by the application of recognized methods of chemical investigation unravel the mysteries tied up in it; but unless we have the climatic and cultural conditions uniform, we cannot hope to secure results that will be of general application.

There have been two methods of attacking the problem: first, the isolation and study of the individual organic compounds existing in the soil, and, second, a study of the hydrolysis products. It is evident that these methods are slow and tedious and unsatisfactory, but on the other hand a study of all the possible combinations of the organic substances existing in the soil appears to be an endless task in the light of our present knowledge. In brief, a complete and thorough knowledge of the organic matter of the soil appears possible only after we have mastered all of the biochemical processes which are characteristic of the fungi, protozoa, and bacteria of the soil, and have a much deeper insight into the chemical constitution of vegetable cells than we have at the present time. Our present knowledge leads us to believe that it is possible to isolate an almost infinite variety of chemical compounds from a soil, the number and variety reaching a limit only when we have isolated all of the compounds which are present in the plants which grew upon the soil, plus those compounds contained in the bodies of bacteria, protozoa, and fungi, plus all of the compounds which may be derived from these compounds under the peculiar soil conditions of decay, oxidation, bacterial action, and the secretions of fungi and living plant roots.

II. EXPERIMENTAL: A STUDY OF THE NITROGEN DISTRIBUTION IN DIFFERENT SOIL TYPES.

A. The Problem.

It has been shown in the historical study above that a number of investigators have studied the organic nitrogen distribution in the soil by applying either Hausmann's (1899) or Van Slyke's (1910, 1911) method of protein analysis. It has been demonstrated by Potter and Snyder that various plots on a single soil type under different fertilizer treatments gave, with Van Slyke's method, essentially the same results.

I have taken up the problem at this point and made a similar study of the organic nitrogen distribution in **different soil types** in an attempt to see whether the forms in which nitrogen occur differ from locality to locality and from soil type to soil type. I am concerned with the problem of distribution of the organic nitrogen in the soils and soil extracts studied.

B. The Material.

The study was made using two peats, one muck, seven mineral surface soils, and one subsoil. All were from the State of Minnesota. The origin and type names are in accordance with the surveys of the Bureau of Soils of the U. S. Department of Agriculture when such surveys were available. Almost all of the soils used are portions of the identical samples employed by Gortner (1916 a, and 1917) in his soil studies. The samples used in this study were all **air dry** soils. The moisture was determined by heating the soils to a temperature of 100° C. for 12 hours and then weighing.

The descriptions of the soils follow:

1. **Calcareous black grass-peat.** This sample was selected from a large bulk sample taken to a depth of 8 inches from a grass bog near the Agricultural Experiment Station Farm, St. Paul. The peat was black and well decomposed. The peat was ground to a powder in a ball mill before using. The air dry material contained 6.40 per cent moisture.

2. **Sphagnum-covered peat.** This sample of very strongly acid peat was prepared from a large bulk sample taken from a bog on the Experimental Farm near Grand Rapids. The superficial layer of sphagnum and shrubs was first removed and a sample of the underlying peat taken to a depth of 8 inches. The peat was poorly decomposed. The sample was prepared by grinding to a powder in a ball mill. The air dry soil contained 5.90 per cent moisture.

3. **Acid "muck" soil.** A sample of this strongly acid soil was obtained from a bog about two miles from the farm of the Agri-

cultural Experiment Station, St. Paul. The sample is a composite of 10 samples taken to a depth of 8 inches lengthwise of the bog. The vegetation of the bog was largely cattails *(Typha spp.)* and rushes *(Scirpus spp.)* The sample contained 5.60 per cent moisture in the air dry condition.

4. **Fargo clay loam.** The sample analyzed consisted of a composite made from 144 borings taken to a depth of 8 inches from a twenty-acre field on the Northwest Sub-station Farm, Crookston. The sample was highly calcareous. This soil type has been described by Mangum and Schroeder (1906). The moisture content of the air dry soil was 3.92 per cent.

5. **Fargo silt loam.** This sample was a composite made from 100 borings to a depth of 6 inches, twenty borings being taken from each of five virgin fields near Nerstrand, Rice County. The sample had a neutral reaction. The soil type has been described by Burke and Kolbe (1909). The air dry soil contained 14.89 per cent moisture.

6. **Carrington silt loam.** This soil is represented by two samples. Each composite consisted of 100 borings to a depth of 6 inches, twenty borings being taken from each of five virgin fields near Nerstrand, Rice County, for Sample number I, and twenty borings being taken from each of five virgin fields near Morristown, Rice County, for Sample number II. Sample I was strongly acid while Sample II was but slightly acid. The soil type is described by Burke and Kolbe (1909). The moisture content of sample I was 6.22 per cent.

7. **Hempstead silt loam.** A composite sample from 36 borings to a depth of 6 inches was taken from 12 plots on the Agricultural Experiment Station Farm, St. Paul. No commercial fertilizer had been applied, but the land had long been under cultivation. The soil type has been described by Smith and Kirk (1914). The sample was strongly acid. The air dry soil contained 3.07 per cent moisture.

8. **Prairie-covered loess.** The sample consisted of 50 borings taken to a depth of one foot, ten borings being taken from each of five virgin fields near Luverne, Rock County. The sample was somewhat calcareous. The area from which the sample was obtained has not been subjected to a detailed soil survey. The air dry soil contained 7.89 per cent moisture.

9. **Forest-covered loess.** This sample was taken from five virgin fields near Caledonia, Houston County, ten borings to a depth of one foot being taken from each field and equal weights from each boring being combined in the composite sample. The sample was strongly acid. The air dry soil had a moisture content of 1.87 per cent.

10. **Hempstead silt loam subsoil.** This was a third foot bulk sample taken from a grove on the farm of the Agricultural Experiment Station, St. Paul. The sample was strongly acid.

C. The Method.

The method of Van Slyke (1911) has been used throughout this investigation because the nitrogen can be separated into a larger number of fractions than when the earlier method of Hausmann (1899) is employed. The different fractions, however, are not listed in the same manner as in the Van Slyke method, for since we are not dealing with pure protein material we cannot correctly speak of arginine, histidine, cystine, and lysine nitrogen.

Van Slyke (1915) has called attention to the fact that his method was devised for the analysis of **pure protein** material and not for a heterogeneous mixture of nitrogen compounds. This fact was not recognized by certain investigators. Grindley and Slater (1915) applied this method to the analysis of feeding stuffs in exactly the same manner as though they were dealing with a pure protein. Potter and Snyder (1915 a) analyzed certain soils and soil extracts and report their fractions as "arginine," "histidine," etc. Although they state (p. 2221):

> It is not thought that nitrogen as found by the Van Slyke method, working with such a complex as the soil, is in reality, all lysine, histidine, etc., nitrogen. It might be said that each group, as found, represents a class of compounds having the particular reaction by which the lysine, histidine, etc., nitrogen respectively are determined.

It is obvious that there are other types of organic materials which will interfere with the nitrogen distribution. It seems very probable that in soils as well as in the material analyzed by Grindley and Slater (1915) there must be many organic nitrogen compounds which have no relation to the protein molecule, such as purine bases, pyrimidine bases, nitrogenous lipins, nitrogenous pigments, as well as other non-protein nitrogenous compounds (**cf.** the list of non-protein nitrogenous compounds actually isolated from the soil as given in the preceding part of this paper). Gortner (1913) states that much valuable **comparative** data can be obtained by the application of Van Slyke's method to the analysis of heterogeneous materials; but it is self evident that no analogy can be drawn between the analysis of pure protein and the analysis of a protein mixed with an unknown amount of foreign nitrogenous compounds. The results of Potter and Snyder (1915 a) are of little value in advancing our knowledge of soil proteins or for comparison with analyses of pure proteins, but are extremely valuable and interesting for comparison between themselves and with other analyses of soils carried out under similar conditions. It must be remembered that all data on similar material is strictly comparable when the same method of analysis is followed.

It is possible that many of the non-protein nitrogenous compounds may be split up during the hydrolysis of heterogeneous material. Gortner (1913) has shown that uric acid nitrogen is distributed in all four of the major fractions after hydrolysis. The ammonia nitrogen amounted to 15.27 per cent, humin nitrogen 35.98 per cent, basic nitrogen 12.97 per cent, and non-basic nitrogen 35.78 per cent. "The humin nitrogen contained no trace of black color and was probably calcium ureate." Probably all of the purines and pyrimidines would behave in a similar manner.

The general method employed in this investigation will be discussed in detail for two soils, a peat and a mineral soil, inasmuch as the experimental conditions vary in minor details with the two types.

1. **The method in detail for a peat soil.** Duplicate samples of 15 grams were hydrolyzed in the presence of hydrochloric acid for 48 hours. The content of calcium oxide was taken into account, and corrections made so that the 100 cc. of hydrochloric acid used was of sufficient concentration to neutralize the lime and at the same time furnish a constant boiling acid. The hydrolysis was carried out in 200 cc. long neck, round bottom Kjeldahl flasks, fitted with modified Hopkins condensers made from a test tube which fit rather loosely into the neck of the flask. By means of this device any error due to products extracted from cork or rubber stoppers was obviated. The flasks were heated to gentle boiling on the same sand bath over an Argand burner, so that the rate of hydrolysis would be as near the same as possible.

After completion of the 48 hour hydrolysis the mixture was evaporated in a Claissen distilling flask under diminished pressure until all the hydrochloric acid possible was driven off. The residue after this distillation was dissolved in 100-150 cc. of water, 100 cc. of 95 per cent alcohol, and an excess of calcium hydroxide suspended in water was added and the ammonia distilled off into standard acid at a temperature of 40-50° C. under a pressure of less than 30 mm., distillation being continued for at least a half hour. The results are listed under "ammonia nitrogen."

The alkaline mixture in the distilling flask was filtered and the precipitate well washed with hot water until free of chlorides. A Kjeldahl determination was made of the filter and its contents, and the results listed under "humin nitrogen."

The filtrate and washings from the humin were acidified with hydrochloric acid and concentrated under diminished pressure to less than 200 cc. To this solution was added 18 cc. of concentrated hydrochloric acid and the whole heated on the water bath until hot. A solution containing 15 grams of phosphotungstic acid was then added and the heating on the water bath continued for an hour. The flask was then set aside in a cool place for 48 hours. The precipitate of the bases was then filtered off and washed as directed by Van Slyke (1911).

The basic phosphotungstates were suspended in 800 cc. of water and brought into solution by the cautious addition of a 50 per cent solution of sodium hydroxide, a few drops of phenolphthalein being added to guard against too great an excess of alkali. The phophotungstic acid was precipitated by the addition of a slight excess of 20 per cent barium chloride, and the barium phosphotungstate was filtered off and washed free of chlorides with hot water.

The filtrate and washings were united, acidified with hydrochloric acid, and concentrated under diminished pressure to a very small volume. After cooling the residue was filtered, washed and made up to 50 cc. volume.

The washed precipitate of barium phosphotungstate and filter were subjected to Kjeldahl determination for any nitrogen that might be held by absorption, adsorption, or occlusion, as was also the filter and contents remaining after the final filtration of the solution containing the basic nitrogen. In all cases some nitrogen was found. This nitrogen is probably derived from the "unadsorbed humin carried down with the basic phosphotungstates" mentioned by Van Slyke (1915, p. 284). Inasmuch as my work was done prior to this publication, I added this nitrogen to the total nitrogen content of the bases instead of to the humin.

During the distillation after kjeldahling this precipitate the cochineal indicator took on a color which made the acid solution appear that complete neutralization might have occurred when in fact it had not. This color change was noticed in every case with the barium phosphotungstate distillate. This made titration difficult, since a new end point had to be established. Gortner and Holm (private communication) have observed a similar color change in the case of fibrin hydrolyzed in the presence of a large excess of formaldehyde. They explain this finding on the assumption that pyridine (or some similar base) is formed which is not easily broken down in the Kjedahl process (cf. Dakin and Dudley, 1914), and which greatly influences the color changes of the indicator when the base is volatalized during the subsequent distillation with alkali. Whether or not this is the cause of the phenomenon observed in my materials cannot be ascertained without further investigation.

In no case did I attempt to separate the basic nitrogen into the usual fractions of "arginine," "cystine," "histidine," and "lysine" nitrogen, because I am not dealing with pure protein. Instead in each case the total nitrogen liberated as ammonia was determined on 25 cc. of the solution containing the bases. This was determined in exactly the same manner that Van Slyke used for the determination of arginine nitrogen. The volume of standard acid neutralized by the ammonia indicated the amount contained in the 25 cc. of solution used. The nitrogen found is listed as "basic nitrogen set free as ammonia by 50 per cent potassium hydroxide." The solution remaining from this determination was used in the estimation of the total nitrogen of the bases. This was performed according to Van Slyke's directions. The quantity of acid neutralized in this determination was added to that neutralized in the basic-nitrogen-set-free-as-ammonia-by-50-per-cent-potassium-hydroxide, thus securing the "total basic nitrogen."

The "amino nitrogen of the bases" was determined in Van Slyke's (1912) apparatus, using 10 cc. portions of the solution.

The filtrate from the bases was treated with sodium hydroxide solution until a slight turbid precipitate of lime was formed, and then cleared immediately by the addition of acetic acid. This was concentrated under diminished pressure and on cooling was made to 200 cc. volume. The solutions were more or less violet in color. "Total nitrogen in the filtrate from the bases" was determined on duplicate portions of 25 cc. each by the method of Kjeldahl. The

digestion was continued for three hours after the solutions were clear, so that the phosphotungstic acid would not interfere with the accuracy of the determination. The "amino nitrogen in the filtrate from the bases" was determined on duplicate portions of 10 cc. each by means of the Van Slyke (1912) apparatus.

2. The method in detail for a mineral soil. Duplicate portions of 250 grams were hydrolyzed in 500 cc. round bottom Kjeldahl flasks for 48 hours on different sand baths. Allowance was always made for the lime content of the soil, and the requisite amount of hydrochloric acid added to insure the presence of a constant boiling acid (sp. gr. 1.115), and a volume of approximately 250 cc. The solutions boiled smoothly and gave no trouble by bumping. **Air dry** soil was used in all cases, but the moisture was determined on a separate portion and all data calculated to the dry basis.

On completion of the hydrolysis each of the two samples was diluted to a 1000 cc. in measuring flasks and allowed to settle for at least 24 hours. The clear solution was then syphoned off and an aliquot of 500 cc. analyzed according to the usual method of Van Slyke. In nearly all cases this solution was straw color due to the presence of ferric salts that had been formed during the hydrolysis. No black color, the usual color of a protein hydrolysate, was observed in any instance.

Another aliquot of 100 cc. was used for the determination of total nitrogen in the solution by making duplicate Kjeldahl determinations on 50 cc. portions. A second aliquot of 100 cc. was used for the determination of "Jodidi numbers" (**cf.** p. 25) when they were determined.

The soil remaining in the measuring flask was washed free from soluble nitrogen with a 1 per cent solution of potassium sulfate by decantation from tall soil beakers, the solution after settling being syphoned off not oftener than twice a day. This method was employed in order to prevent the clay from forming a colloidal suspension. Distilled water alone would remove all electrolytes and allow a portion of the clay to remain in the solution in colloidal suspension. It is known that suspensions of finely divided clay carry a negative charge in pure water. Since it is necessary for the complete precipitation of colloids to have some electrolyte present it was decided to use a 1 per cent solution of potassium sulfate. The negatively charged colloid was thus precipitated by the positive ions of the potassium sulfate solution and at the same time the salt would not interfere with the subsequent Kjeldahl determination.

A concrete example of the thoroughness of this washing may well be given. It will be noted that 700 cc. of the original hydrolysate was syphoned off for the different analyses. This left a total volume of 300 cc. of residue and solution to be washed by decantation with 1 per cent potassium sulfate. By the methods of calculation given in the following paragraphs it was found that the remaining solution contained 0.1089 gram of nitrogen. If three-fourths of the wash solution is removed each time, there will remain

in the solution at the end of the fourth washing approximately 0.0004 gram of the original nitrogen. Actual Kjeldahl determinations were made on 250 cc. portions from the fourth washing in the case of duplicates from the same soil, and the results indicated that 0.0006 gram of nitrogen still remained in the solution. Since this was within experimental error of the theoretical value, the method of washing by decantation was followed in all the subsequent work with mineral soils, or those which had mineral soils added previous to the analysis.

The residue from the hydrolyzed soil was evaporated to dryness on the steam bath in an evaporating dish, then further dried at about 110° C. After cooling, this dry soil was passed through a 1 mm. sieve and after being thoroughly sampled, duplicate nitrogen determinations were made on 15 gram portions and the total nitrogen remaining in the soil calculated. These results are listed as "insoluble humin nitrogen in the soil." The weight of the dry soil divided by the average specific gravity (2.6) represented the actual volume occupied by this soil residue. The total volume of the hydrolysate minus the volume occupied by the insoluble residue gives the actual volume of the soil solution.

Since the analysis was made on 500 cc. of the soil solution it was necessary to recalculate the total "insoluble humin nitrogen in the soil" in order to determine the amount of this humin nitrogen actually belonging to the aliquot analyzed.

The total nitrogen belonging to the solution analyzed was found by taking the sum of the total nitrogen in the solution and the above insoluble humin nitrogen. Knowing the total nitrogen content of the soil before hydrolysis and the total nitrogen in solution, the per cent of the total nitrogen in solution after hydrolysis can be determined.

The 500 cc. aliquot was concentrated under reduced pressure until the hydrochloric acid was practically removed and the ammonia nitrogen determined in the manner outlined under the peat analysis.

The "humin" fraction precipitated by the calcium hydroxide was almost colorless or light yellow, due to the iron salts contained in it. This bulky precipitate was always washed by decantation after the method above described, except that distilled water was used, the united washings being concentrated to 200 cc. or less for the precipitation of the basic nitrogen.

It was found necessary to use 35 grams of phosphotungstic acid for the precipitation of the bases. The remainder of the analysis was carried out as directed under peats.

3. The method for determination of "Jodidi numbers." A 100 cc. portion of the clear hydrolysate was concentrated under reduced pressure and the ammonia nitrogen determined in the usual manner. The residue remaining in the flask after this determination was dissolved in concentrated hydrochloric acid and phosphotungstic acid added.* After standing the usual length of time the precipitate was filtered off and the total nitrogen deter-

*It will be noted that no "humin" fraction is separated. In that respect the "Jodidi numbers" differ from Hausmann numbers.

mined on the filter and contents by the Kjeldahl method and listed as "basic N." The filtrate from the above precipitate was concentrated and made to 300 cc. volume. Duplicate determinations were made on 100 cc. portions, and the total nitrogen listed as nitrogen in the filtrate from the "bases."

4. **The determination of nitrogen.** Nitrogen was determined on the soils and soil extracts in the usual manner, using 25-35 cc. H_2SO_4, 10 gm. K_2SO_4, and a crystal of $CuSO_4$. All titrations were made with N/14 acid and alkali so that the figures obtained represented milligrams of nitrogen without necessitating a calculation.

D. The Analytical Data.

The essential data which have already been published on the soils studied are shown in Table I.

Table I.—Certain analytical data for the soils used in this paper. Data of Gortner (1916 a).

	Per cent moisture in air dry soil.	Per cent total nitrogen in soil (dry basis).	Per cent organic carbon in soil (dry basis).	Per cent carbonate CO_2 in soil (dry basis).	Per cent "humus" in soil (dry basis).	Ratio C/N.
Calcareous black grass-peat	6.40	2.940	42.81	0.600	28.71	14.56
Sphagnum-covered peat	5.90	2.000	49.32	none	32.91	24.66
Acid "muck" soil....	5.60	1.340	14.58	none	7.14	10.88
Fargo clay loam....	3.92	0.250	2.678	2.360	2.66	10.72
Fargo silt loam.....	14.89	0.823	10.02	0.200	9.91	12.17
Carrington silt loam.	6.22	0.371	4.733	0.090	4.95	12.76
Hempstead silt loam	3.07	0.256	3.373	0.020	3.61	13.17
Prairie-covered loess	7.89	0.301	3.704	0.240	3.40	12.30
Forest-covered loess	1.87	0.128	1.638	0.120	1.79	12.79

1. **Analysis of "fibrin from blood" hydrolyzed in the presence of 100 grams ignited subsoil.** This analysis was conducted in order to ascertain if possible the effect of soil minerals upon the hydrolysis of a pure protein. Fibrin was selected because it was from a sample already analyzed (Gortner 1916 c). The subsoil was first ignited to redness in a muffle furnace for an hour, in order to drive off all the organic matter, and subsequently cooled in a desiccator.

Duplicate portions of five grams fibrin and 100 grams ignited subsoil were hydrolyzed in the presence of an excess of hydrochloric acid. Upon the application of heat, fumes of hydrogen chloride were evolved for some time. The analysis was conducted like that of the mineral soils, excepting that a 600 cc. aliquot was used for the analysis, this amount of solution being equivalent to three grams of fibrin. After making the ammonia determination the humin precipitate was washed by decantation in the usual manner and the filtrate made to 250 cc. volume. The first wash solu-

tion from the humin precipitate had a characteristic light blue color in each case. Duplicate Kjeldahl determinations were made on 25 cc. portions of this solution and the results listed as total-nitrogen-in-the-filtrate-from-humin. The remaining 200 cc. solution was used for the precipitation of the bases and the subsequent analysis, but of course the results were calculated on the basis of the total volume. The total nitrogen belonging to the aliquot analyzed was determined by adding the nitrogen obtained as ammonia, humin, and total-nitrogen-in-the-filtrate-from-humin to the insoluble-humin-nitrogen-in-the-soil. The filtrate-from-the-bases was made to 250 cc. volume. Twenty-five grams of phosphotungstic acid was used for the precipitation of the bases. The nitrogen retained by the barium phosphotungstate was 0.0022 gram for Sample I and 0.0020 gram for Sample II.

The experimental data showing the grams of nitrogen found and per cent of total nitrogen are given in Table II.

Table II.—Nitrogen distribution in three grams of Merck's "fibrin from blood" hydrolyzed in the presence of 100 grams of ignited subsoil.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.4577	0.4591	.....	.....	.....
Ammonia N	0.0457	0.0455	9.98	9.91	9.95
Insoluble humin N in soil	0.0125	0.0130	2.73	2.83	2.78
Humin N pptd. by $Ca(OH)_2$	0.0220	0.0221	4.81	4.81	4.81
Basic N	0.1219	0.0995	26.63	21.68	24.15
Arginine N	0.0598	0.0440	13.07	9.58	11.32
Histidine N	none	none	.....	.....	none
Lysine N	0.0597	0.0531	13.04	11.57	12.30
Cystine N	.....	.....	.....	.....	0.5[1]
Amino N in bases	0.0775	0.0746	16.93	16.25	16.59
N in filtrate from bases	0.2725	0.2909	59.54	63.36	61.45
Amino N in filtrate from bases	0.2671	0.2702	58.36	58.85	58.61
Non-amino N in filtrate from bases	0.0054	0.0207	1.18	4.51	2.84
Total N regained	0.4746	0.4710	103.69	102.59	103.14

[1]From data of Gortner (1916 c).

Table III shows a comparison of these analyses with other analysis of the same sample of fibrin hydrolyzed alone, and in the presence of three times its weight of cellulose (data of Gortner 1916 c).

Differences between these analyses, together with differences between duplicates in each series of analysis, and data showing "maximum" and "average" experimental differences to be expected are given in Table IV.

These comparisons will be considered in detail under "Discussion" in the latter part of this paper.

Table III.—Comparative analyses of three grams of Merck's "fibrin from blood" hydrolyzed alone and in the presence of carbohydrate and of ignited subsoil.*

	3 gm. fibrin hydrolyzed alone. Data of Gortner (1916 c)	3 gm. fibrin hydrolyzed in the presence of 9 gm. of cellulose. Data of Gortner (1916 c)	3 gm. fibrin hydrolyzed in the presence of 100 gm. of ignited subsoil. (Average data of Table II)
	Per cent total nitrogen	Per cent total nitrogen	Per cent total nitrogen
Ammonia N	10.15	9.85	9.95
Humin N	2.83	7.72	7.59
Arginine N	10.91	8.56	11.32
Histidine N	4.36	4.86	none
Lysine N	12.05	11.04	12.30
Cystine N	0.51	0.71	0.51
Amino N in filtrate from bases	55.43	52.02	58.61
Non-amino N in filtrate from bases	2.51	3.91	2.84
Total N regained	98.75	98.67	103.14

*The three gram portion contained approximately 0.4550 gm. nitrogen.

Table IV.—Difference between duplicate analyses (due to experimental errors), the differences apparently due to the addition of carbohydrate and of ignited subsoil, as well as Van Slyke's "maximum" and "average" differences to be expected.

	Per cent difference between duplicates of fibrin alone (Data of Gortner 1916 c).	Per cent difference between duplicates of fibrin and 3 times its weight of carbohydrate (Data of Gortner 1916 c).	Per cent difference between duplicates of fibrin and 100 gm. of ignited subsoil.	Average per cent difference between fibrin hydrolyzed alone and with 3 times its weight of carbohydrate. (Data of Gortner 1916 c).	Average per cent difference between fibrin hydrolyzed alone and in the presence of 100 gm. of ignited subsoil.	Van Slyke's (1911) experimental differences	
						"Maximum" per cent	"Average" per cent
Ammonia N	0.01	0.48	0.07	—0.30	—0.20	0.37	0.12
Humin N	0.11	0.25	0.10	+4.89	+4.76	0.39	0.20
Arginine N	0.73	0.86	3.49	—2.35	+0.41	1.27	0.73
Histidine N	0.07	0.66	none	+0.50	—4.36	2.14 (0.93)[1]	0.79
Lysine N	0.08	0.11	1.47	—1.01	+0.25	1.23	0.61
Cystine N	0.10	0.00	....	+0.20	.....	0.11	0.05
Amino N in filtrate from bases	2.12	2.15	0.49	—3.41	+3.18	1.60 (0.60)[1]	0.63
Non-amino N in filtrate from bases	0.27	0.40	3.23	+1.40	+0.33	1.20	0.68

[1]The figure in parentheses represents the second greatest difference between duplicates observed by Van Slyke (1911).

2. Calcareous black grass-peat. Duplicate samples of 15 grams were hydrolyzed for 48 hours in the presence of 100 cc. constant boiling hydrochloric acid, and the analysis conducted as described under the method for a peat soil.

In the precipitation of the phosphotungstic acid with barium chloride, it was difficult to determine when the precipitation was complete. On addition of barium chloride a precipitate formed and the pink color slowly disappeared. Both sodium hydroxide and barium chloride were repeatedly added. The change in color occurred several times during a half-hour. At all times test portions gave immediate granular precipitates with both sodium sulfate and barium chloride. This indicated that we could not depend upon the test with sodium sulfate to determine when all the phosphotungstic acid was precipitated. After the addition of a large amount of barium chloride the solution was allowed to stand several hours in order to allow equilibrium to be established. At the end of this time test portions gave a heavy precipitate with sodium sulfate, but no action with barium chloride. In all subsequent work, both with peat and mineral soils, the addition of barium chloride to the solution of the phosphotungstates was extended over an hour or more and the precipitate then allowed to settle over night.

This barium phosphotungstate precipitate was washed thoroughly by decantation with hot water until free of chlorides. This operation was carried out in the original beaker, and in the case of many mineral soils required from 500 to 1000 cc. to remove all chlorides. The washed precipitate of barium phosphotungstate and filter on kjeldahling gave 0.0021 gram nitrogen retained by Sample I and 0.0055 gram by Sample II.

The experimental data showing the grams of nitrogen and per cent of total nitrogen are given in Table V.

Table V.—Nitrogen distribution in calcareous black grass-peat.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.4412	0.4412	.....	.....	.....
Ammonia N	0.0833	0.0867	18.88	19.65	19.26
Humin N	0.1145	0.1155	25.95	26.18	26.07
Basic N	0.0494	0.0441	11.20	10.00	10.60
Basic N set free as NH_3 by 50% KOH	0.0150	0.0139	3.40	3.15	3.27
Basic N not set free as NH_3 by 50% KOH	0.0344	0.0302	7.80	6.85	7.33
Amino N of bases	0.0309	0.0251	7.00	5.69	6.35
Non-amino N of bases	0.0185	0.0190	4.19	4.31	4.25
N in filtrate from bases	0.1932	0.1810	43.79	41.02	42.40
Amino N in filtrate from bases	0.1810	0.1672	41.02	37.90	39.46
Non-amino N in filtrate from bases	0.0122	0.0138	2.77	3.12	2.94
Total N regained	0.4404	0.4273	99.82	96.85	98.33

3. **Sphagnum-covered peat.** Duplicate 15 gram samples were hydrolyzed in the usual manner. It was found in the ammonia determination that all of the ammonia nitrogen could not be driven off in a half-hour when the volume of solution was large and a bulky precipitate of iron and aluminum hydroxides was present. Continued distillation for a further half-hour in this case gave additional ammonia nitrogen amounting to 0.0060 gram. In all subsequent work with both peats and soils the volume was kept smaller by the use of a more concentrated suspension of calcium hydroxide.

The calcium hydroxide precipitate containing the "humin" nitrogen was difficult to digest in the subsequent Kjeldahl determination, due to the large amount of carbonaceous organic material present. From 100-200 cc. of sulfuric acid were required for the digestion. After digestion the material was transferred to a 1000 cc. flask and 250 cc. portions used for the distillation. It was often necessary to heat the flask on a sand bath to prevent severe bumping. This trouble was obviated in later work by adding zinc dust instead of granulated zinc to the distilling flask. The nitrogen retained by the barium phosphotungstate amounted to 0.0009 gram in Sample I, and 0.0013 gram in Sample II.

The experimental data showing the grams of nitrogen and per cent of total nitrogen are given in Table VI.

Table VI.—Nitrogen distribution in sphagnum-covered peat.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.3000	0.3000	.....	.....	.....
Ammonia N	0.0659	0.0737	21.97	24.57	23.27
Humin N	0.0779	0.0804	25.96	26.80	26.38
Basic N	0.0281	0.0303	9.37	10.10	9.73
Basic N set free as NH_3 by 50% KOH	0.0091	0.0088	3.03	2.93	2.98
Basic N not set free as NH_3 by 50% KOH	0.0190	0.0215	6.32	7.17	6.75
Amino N of bases	0.0171	0.0145	5.70	4.83	5.26
Non-amino N of bases	0.0110	0.0158	3.67	5.27	4.47
N in filtrate from bases	0.1396	0.1184	46.53	39.46	43.00
Amino N in filtrate from bases	0.1292	0.1135	43.06	37.83	40.45
Non-amino N in filtrate from bases	0.0104	0.0049	3.47	1.63	2.55
Total N regained	0.3115	0.3028	103.83	100.93	102.38

4. **Acid "muck" soil** One 25 gram sample was hydrolyzed in the presence of 125 cc. concentrated hydrochloric acid for 48 hours. The ammonia nitrogen was determined as usual, and the precipitate of calcium hydroxide containing the "humin" was washed in a tall soil beaker in the manner described under the method for a mineral soil. After digestion of this precipitate, the material in the Kjeldahl flask was diluted to 500 cc. and duplicate distillations made on 250 cc. portions. The bases were precipitated with 15 grams phosphotungstic acid. The total nitrogen retained by the barium phosphotungstate was 0.0022 gram. The solution of the filtrate-from-the-bases was made to a volume of 250 cc.

The experimental data showing the grams of nitrogen and per cent of total nitrogen are found in Table VII.

Table VII.—Nitrogen distribution in an acid "muck" soil.

	Grams nitrogen	Per cent of total nitrogen
Total N	0.3350	.....
Ammonia N	0.0653	19.49
Humin N	0.0925	27.61
Basic N	0.0454	13.55
Basic N set free as NH_3 by 50% KOH	0.0104	3.10
Basic N not set free as NH_3 by 50% KOH	0.0350	10.45
Amino N of bases	0.0306	9.13
Non-amino N of bases	0.0148	4.42
N in filtrate from bases	0.1300	38.81
Amino N in filtrate from bases	0.1133	33.82
Non-amino N in filtrate from bases	0.0167	4.99
Total N regained	0.3332	99.46

5. Fargo clay loam. Duplicate portions of 250 grams were hydrolyzed for 48 hours. The "humin" precipitate required 100 cc. sulfuric acid for the digestion. After digestion the material was transferred to a 500 cc. flask and 250 cc. portions used for the distillation. This method was followed subsequently with the "humin" nitrogen determination of all the mineral soils.

The filtrate from "humin" was of a sirupy consistency in each case. The first addition of 15 grams of phosphotungstic acid did not entirely precipitate the bases. Five gram portions were added from time to time until a total of 50 grams had been used. After standing the usual length of time the precipitate of the bases was filtered off, but even then the wash water caused the formation of a small additional precipitate in the filtrate. After warming on the steam bath this final solution was perfectly clear, and on standing over night, only a trace of precipitate was formed so the precipitation was considered complete. It appears probable that a portion of this precipitate is due to the formation of inorganic phosphotungstates which consume a very large amount of the phosphotungstic acid, for if all of this precipitate had consisted of basic nitrogen compounds the amount of nitrogen recovered should have been greater than the amount which was actually found. In all the subsequent work 35 grams of phosphotungstic acid was used for the precipitation of the bases in the hydrolysates from mineral soils.

The phosphotungstate precipitate dissolved very slowly in the sodium hydroxide as did all other phosphotungstic acid precipitates of the mineral soils studied. A Kjeldahl determination of the barium phosphotungstate gave 0.0026 gram nitrogen. The solution containing the nitrogen of the bases was made up to 100 cc. volume.

During the concentration of the filtrate from the bases so much precipitate separated, that this was filtered off and the solution made up to 300 cc. volume. The salt remaining was dissolved in water and also made up to a volume of 300 cc. Aliquot portions were taken from each solution and combined for the different determinations.

The experimental data giving the grams of nitrogen and per cent of total nitrogen are found in Table VIII.

Table VIII.—Nitrogen distribution in Fargo clay loam.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.3368	0.3338	.....	.....	.....
Ammonia N	0.0788	0.0821	23.40	24.60	24.00
Insoluble humin N in soil.	0.0948	0.0948	28.15	28.40	28.27
Humin N pptd. by $Ca(OH)_2$	0.0352	0.0266	10.45	7.97	9.21
Basic N	.....[1]	0.0320	.....	9.58	.....
Basic N set free as NH_3 by 50% KOH	.....	0.0108	.....	3.23	3.23
Basic N not set free as NH_3 by 50% KOH	.....	0.0212	.....	6.35	6.35
Amino N of bases	.....	0.0215	.....	6.44	.....
Non-amino N of bases	.....	0.0105	.....	3.14	.....
N in filtrate from bases	.....	0.1092	.....	32.71	.....
Amino N in filtrate from bases	.....	0.1027	.....	30.77	30.77
Non-amino N in filtrate from bases	.....	0.0065	.....	1.95	1.95
Total N regained	.....	0.3447	.....	103.26	103.77

[1] Entire sample lost during precipitation of bases.

6. Fargo silt loam. Two 125 gram portions were hydrolyzed for 48 hours with 500 cc. hydrochloric acid (sp. gr. 1.18). During the first few hours large amounts of hydrogen chloride were evolved.

The resulting hydrolysates from the two flasks were combined and diluted to 2 liters. After settling, a 1 liter portion was syphoned off and analyzed by the usual method. Two 100 cc. portions were used for the determination of total nitrogen in solution, and another portion of 200 cc. was used for determination of the "Jodidi numbers." The nitrogen retained by the barium phosphotungstate was 0.0091 gram. The solution containing the basic nitrogen was made to 100 cc. volume and that containing the nitrogen in the filtrate from the bases to 300 cc. volume.

The experimental data showing grams of nitrogen and per cent of total nitrogen are given in Table IX.

Table IX.—Nitrogen distribution in Fargo silt loam.

	Grams nitrogen	Per cent of total nitrogen
Total N	0.9238	.....
Ammonia N	0.2454	26.56
Insoluble humin N in soil	0.2118	22.93
Humin N pptd. by $Ca(OH)_2$	0.0301[1]	3.26
Basic N	0.1119	12.11
Basic N set free as NH_3 by 50% KOH	0.0296	3.20
Basic N not set free as NH_3 by 50% KOH	0.0823	8.91
Amino N of bases	0.0695	7.52
Non-amino N of bases	0.0424	4.59
N in filtrate from bases	0.3246	35.14
Amino N in filtrate from bases	0.3015	32.64
Non-amino N in filtrate from bases	0.0231	2.50
Total N regained	.....	100.00[2]

[1] By difference. [2] Calculated.

7. **Carrington silt loam.** Sample I (Nerstrand origin). The hydrolysis of 250 grams was carried out according to the method described under Fargo silt loam. The "humin" nitrogen was determined as outlined under Fargo clay loam. Sample II (Morristown origin) was hydrolyzed and the same methods of analysis employed throughout as was applied to Sample I. The nitrogen retained by the barium phosphotungstate precipitate was 0.0028 gram in Sample I and 0.0025 gram in Sample II. In both samples the solutions containing the bases and filtrate from the bases were made to the same dilution as under Fargo silt loam.

The experimental data showing grams of nitrogen and per cent of total nitrogen are given in Table X.

Table X.—Nitrogen distribution in Carrington silt loam.

	Sample No. I		Sample No. II	
	Grams nitrogen	Per cent of total nitrogen	Grams nitrogen	Per cent of total nitrogen
Total N	0.5124	.....	0.4920	.....
Ammonia N	0.1463	28.55	0.1381	28.07
Insoluble humin N in soil	0.1324	25.84	0.1210	24.59
Humin N pptd. by $Ca(OH)_2$	0.0304	5.93	0.0326[2]	6.63
Basic N	0.0600	11.71	0.0725	14.74
Basic N set free as NH_3 by 50% KOH	0.0172	3.36	0.0156	3.17
Basic N not set free as NH_3 by 50% KOH	0.0428	8.35	0.0569	11.57
Amino N of bases	0.0412	8.04	0.0393	7.99
Non-amino N of bases	0.0188	3.67	0.0332	6.75
N in filtrate from bases	0.1305	25.47	0.1390	28.25
Amino N in filtrate from bases	.....[1]	.....	0.1272	25.85
Non-amino N in filtrate from bases	.....	.....	0.0118	2.40
Total N regained	0.4996	97.50	0.5032	102.28

[1] Solution lost at this point.
[2] Result calculated from the digestion of one-half the precipitate.

8. **Hempstead silt loam.** Duplicate 250 gram samples were hydrolyzed for 48 hours. The "humin" was washed and the determination carried out as described under mineral soils. The only explanation which occurs for the higher ammonia nitrogen of one hydrolysate is that Sample I must have been heated more strongly during hydrolysis. From unpublished experiments conducted at this Station it has been found that when a pure protein is hydrolyzed under vigorous boiling, a greater amount of ammonia nitrogen is invariably obtained than when hydrolyzed under slow boiling conditions. This observation was made subsequently to my own, so that the importance of the rate of boiling was not known at the time of doing my work.

Only 25 grams of phosphotungstic acid were added for the precipitation of the bases. After standing the solution gave a further precipitate on addition of more phosphotungstic acid. It was concluded, however, that the organic bases were entirely precipitated, for on washing the precipitate with the phosphotungstic acid wash water no additional precipitate formed. The nitrogen determination gave 0.0011 gram retained by the barium phosphotungstate. The solution containing the filtrate from the bases was made to 300 cc. volume.

The experimental data giving the grams of nitrogen and per cent of total nitrogen will be found in Table XI.

Table XI.—Nitrogen distribution in Hempstead silt loam.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.3416	0.3485	.....	.....	.....
Ammonia N	0.1042	0.0989	30.50	28.38	29.44
Insoluble humin N in soil	0.0981	0.0920	28.72	26.40	27.56
Humin N pptd. by $Ca(OH)_2$	0.0164	.....[1]	4.80	.....	4.80
Basic N	0.0389	.....	11.39	.....	.....
Basic N set free as NH_3 by 50% KOH	0.0078	.....	2.28	.....	2.28
Basic N not set free as NH_3 by 50% KOH	0.0311	.....	9.10	.....	9.10
Amino N of bases	0.0254	.....	7.44	.....	.....
Non-amino N of bases	0.0135	.....	3.95	.....	.....
N in filtrate from bases	0.0960	.....	28.10	.....	.....
Amino N in filtrate from bases	0.0839	.....	24.56	.....	24.56
Non-amino N in filtrate from bases	0.0121	.....	3.54	.....	3.54
Total N regained	0.3536	.....	103.51	.....	101.29

[1] Solution lost at this point.

9. Prairie-covered loess. In Sample I, 250 grams were hydrolyzed in the presence of 250 cc. of constant boiling hydrochloric acid for 48 hours. The hydrolysate on cooling was diluted to 1000 cc. and a 500 cc. portion was syphoned off and used for the analysis. In Sample II, two 125 gram samples were hydrolyzed in the same manner as outlined under Fargo silt loam (500 cc. constant boiling hydrochloric acid to 125 grams soil). The dilution and aliquot used for analysis were also the same. The nitrogen retained by the barium phosphotungstate amounted to 0.0020 gram in Sample I, and 0.0072 gram in Sample II. The solution containing the bases was diluted to 100 cc. in both samples.

The experimental data showing grams of nitrogen found and per cent of total nitrogen are given in Table XII.

Table XII.—Nitrogen distribution in prairie-covered loess.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.3907	0.4012	.....	.....	.....
Ammonia N	0.1223	0.1194	31.30	29.76	30.53
Insoluble humin N in soil	0.0922	0.1007	23.60	25.10	24.35
Humin N pptd. by $Ca(OH)_2$	0.0198	0.0213	5.07	5.31	5.19
Basic N	0.0444	0.0562	11.36	14.01	12.68
Basic N set free as NH_3 by 50% KOH	0.0100	0.0132	2.56	3.29	2.92
Basic N not set free as NH_3 by 50% KOH	0.0344	0.0430	8.80	10.72	9.76
Amino N of bases	0.0271	0.0322	6.93	8.03	7.48
Non-amino N of bases	0.0173	0.0240	4.43	5.98	5.20
N in filtrate from bases	0.1152	0.1128	29.49	28.12	28.80
Amino N in filtrate from bases	0.1044	0.1033	26.72	25.75	26.24
Non-amino N in filtrate from bases	0.0108	0.0095	2.76	2.37	2.56
Total N regained	0.3939	0.4104	100.82	102.30	101.56

10. Forest-covered loess. In Sample I, 300 grams of soil were hydrolyzed and diluted in the same manner as Sample I of prairie-covered loess. In Sample II, 300 grams of soil were hydrolyzed under the same conditions as Sample II of prairie-covered loess. The nitrogen retained by the barium phosphotungstate was 0.0024 gram in Sample I, and 0.0023 gram in Sample II. The solution containing the nitrogen of the bases was diluted to 100 cc. in both samples and the solutions containing the total nitrogen of the filtrates were made to a volume of 300 cc.

It is observed that the volume of acid used in the hydrolysis had little effect on the proportion of the different fractions. The only observed difference is in the insoluble humin nitrogen retained by the soil residue, and this is slightly larger in Sample II, which was hydrolyzed in the presence of the greatest excess of acid. In connection with this it must also be noted that there is a somewhat larger quantity of nitrogen in solution in Sample II than in Sample I. Much the same results are shown with the prairie-covered loess. All increases or decreases in the various fractions due to the greater excess of acid may well be considered to be within the experimental error.

The experimental data showing grams of nitrogen found and per cent of total nitrogen are given in Table XIII.

Table XIII.—Nitrogen distribution in forest-covered loess.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.2224	0.2362	.....	.....	.....
Ammonia N	0.0646	0.0669	29.05	28.32	28.69
Insoluble humin N in soil	0.0574	0.0662	25.81	28.03	26.92
Humin N pptd. by $Ca(OH)_2$	0.0140	0.0080[1]	6.29	3.30	4.84
Basic N	0.0316	0.0325	14.21	13.76	13.98
Basic N set free as NH_3 by 50% KOH	0.0074	0.0096	3.33	4.06	3.69
Basic N not set free as NH_3 by 50% KOH	0.0242	0.0229	10.88	9.70	10.29
Amino N of bases	0.0175	0.0172	7.87	7.28	7.57
Non-amino N of bases	0.0141	0.0153	6.34	6.48	6.41
N in filtrate from bases	0.0621	0.0626	27.92	26.50	27.21
Amino N in filtrate from bases	0.0585	0.0568	26.30	24.05	25.17
Non-amino N in filtrate from bases	0.0036	0.0058	1.62	2.45	2.04
Total N regained	0.2297	.....	103.28	100.00[2]	101.64

[1] By difference. [2] Calculated.

11. Sphagnum-covered peat hydrolyzed in the presence of nine times its weight of a mineral subsoil. Duplicate 10 gram samples were hydrolyzed in the presence of 90 grams subsoil with constant boiling hydrochloric acid for 48 hours. The hydrolysate was concentrated as much as possible and ammonia nitrogen determined on the entire mixture by distillation with an excess of calcium hydroxide for one hour.*

*This was the first attempt to determine the nitrogen fractions in the presence of a mineral soil. For certain reasons later analyses have already been reported in this paper. The analyses as reported in this paper are by no means in chronological order, which may explain seeming inconsistencies.

The residue remaining after hydrolysis was washed free of chlorides on ordinary funnels. It was then digested in three Kjeldahl flasks using a total of 30 grams potassium sulfate and 360 cc. sulfuric acid. After digestion the material was transferred to a 1000 cc. flask, made to volume, and the nitrogen content determined as described under sphagnum-covered peat. It will be observed that the total "humin" nitrogen is determined here instead of being reported in two separate fractions as was done in the case of all other analyses containing mineral soil.

The combined filtrate and washings from the "humin" precipitate were concentrated in the usual manner and made to 200 cc. volume. Duplicate Kjeldahl determinations were made on 25 cc. portions of this solution and the results listed, as total nitrogen-in-the-filtrate-from-humin. The remaining 150 cc. portion was used for the precipitation of the bases, the subsequent procedure being completed as described in the "fibrin from blood" analysis. The barium phosphotungstate precipitate retained 0.0016 gram of nitrogen in Sample I, and 0.0053 gram in Sample II.

In the second sample the combined filtrate and washings from the phosphotungstic acid precipitate of the bases were brought to near the neutral point with 50 per cent sodium hydroxide and a small amount of acetic acid added at once. After the addition of the acid it seemed possible that the neutral point had not been reached the first time, so more sodium hydroxide was added until the neutral point was just passed and acetic acid again added. The resulting solution was placed in a double-necked distilling flask and an attempt made to concentrate the solution under diminished pressure. Frothing was so intense that it was impossible to effect any concentration. When alcohol was added the distillation continued quietly as long as any alcohol was present, but after that the frothing continued. The mixture behaved like a concentrated soap solution. The solution was finally concentrated in an evaporating dish over a water-bath and the resulting solution made to 300 cc. volume. On shaking the solution frothed very badly.

The experimental data showing the grams of nitrogen found and the per cent of total nitrogen are given in Table XIV.

A comparison between these analyses and those of the peat hydrolyzed alone is made in Table XV, the data of the peat hydrolyzed alone being taken from Table VI, and recalculated from a 15 gram basis to a 10 gram basis.

Table XIV.—Nitrogen distribution in sphagnum-covered peat hydrolyzed in the presence of nine times its weight of a mineral subsoil.

	Grams nitrogen		Per cent of total nitrogen		
	I	II	I	II	Av.
Total N	0.2441	0.2441	.....	.....	.....
Ammonia N	0.0625	0.0621	25.60	25.44	25.52
Humin N	0.0658	0.0756[1]	26.96	.....[1]	26.96
Basic N	0.0268	0.0374[1]	10.98	.....[1]	10.98
Basic N set free as NH_3 by 50% KOH	0.0095	0.0105	3.89	4.30	4.09
Basic N not set free as NH_3 by 50% KOH	0.0173	.....	7.09	.....	.....
Amino N of bases	0.0177	0.0179	7.25	7.33	7.29
Non-amino N of bases	0.0091	.....	3.73	.....	.....
N in filtrate from bases	0.0954	0.0948	39.08	38.84	38.96
Amino N in filtrate from bases	0.0836	0.0815	34.25	33.39	33.82
Non-amino N in filtrate from bases	0.0118	0.0133	4.83	5.45	5.14
Total N regained	0.2505	.....	102.62	.....	102.42

[1] These results are evidently incorrect and in the "average" column the figures from the first column only are used.

Table XV.—Comparative analyses of sphagnum-covered peat hydrolyzed alone and in the presence of nine times its weight of a mineral subsoil.

	Grams Nitrogen			Apparent distribution of N in subsoil in per cent of total nitrogen
	Peat	Peat + Subsoil[1]	Increase (+) or Decrease(—)	
Total N	0.2000	0.2441	+0.0441	.....
Ammonia N	0.0466	0.0623	+0.0157	35.60
Humin N	0.0527	0.0658	+0.0131	29.71
Basic N	0.0195	0.0268	+0.0073	16.55
Basic N set free as NH_3 by 50% KOH	0.0060	0.0100	+0.0040	9.07
Basic N not set free as NH_3 by 50% KOH	0.0135	0.0168	+0.0033	7.48
Amino N of bases	0.0105	0.0178	+0.0073	16.55
Non-amino N of bases	0.0090	0.0090	.....	.....
N in filtrate from bases	0.0860	0.0951	+0.0091	20.64
Amino N in filtrate from bases	0.0776	0.0825	+0.0049	11.11
Non-amino N in filtrate from bases	0.0084	0.0126	+0.0042	9.52
Total N regained	0.2048	0.2500	+0.0452	102.50

[1] Ninety gm. of subsoil contained 0.0441 gm. of soil nitrogen.

12. Sphagnum-covered peat hydrolyzed in the presence of metallic tin. This peat was hydrolyzed in the presence of a reducing agent because it was thought that the amount of "humin" nitrogen would be reduced, for according to Samuely (1902) the formation of this dark colored product is due to an oxidative process. Hlasiwetz and Habermann (1871 and 1873) hydrolyzed protein with hydrochloric acid in the presence of stannous chloride in order that the solution should remain colorless. Cohn (1896-97

and 1898-99) believed that the use of a reducing agent was not essential, but according to Otori (1904) this is a mistake.

It is perhaps significant that the "humin" nitrogen was reduced to 3.90 per cent by the presence of a reducing solution. It is not known whether there was sufficient tin present to maintain a reducing solution throughout the hydrolysis inasmuch as the ferric iron in the peat would have an oxidizing action on the stannous salt. The sample was known to contain iron but the amount was not determined.

Duplicate 15 gram samples were hydrolyzed with 100 cc. hydrochloric acid (sp. gr. 1.115) for 48 hours in the presence of five and ten grams of tin respectively. The tin was first partially dissolved in the acid before the samples of peat were added. The determination of "humin" nitrogen was carried out as directed under sphagnum-covered peat excepting that the digested material was diluted to 500 cc. instead of to 1 liter. The nitrogen retained by the barium phosphotungstate was 0.0023 gram in Sample I, and 0.0047 gram in Sample II. The solution containing the filtrate from the bases was diluted to a volume of 300 cc. The experimental data giving the grams of nitrogen found and the per cent of total nitrogen are given in Table XVI.

Table XVI.—Nitrogen distribution in sphagnum-covered peat hydrolyzed in the presence of metallic tin.

	Grams nitrogen		Per cent of total nitrogen		
	I 5 gm. tin	II 10 gm. tin	I	II	Av.
Total N	0.3000	0.3000	.....	.....	.....
Ammonia N	0.0633	0.0578	21.10	19.27	20.18
Humin N	0.0699	0.0650	23.30	21.67	22.48
Basic N	0.0320	0.0417	10.67	13.90	12.28
Basic N set free as NH_3 by 50% KOH	0.0099	0.0100	3.30	3.33	3.31
Basic N not set free as NH_3 by 50% KOH	0.0221	0.0317	7.37	10.57	8.97
Amino N of bases	0.0193	0.0219	6.43	7.30	6.86
Non-amino N of bases	0.0127	0.0198	4.23	6.60	5.41
N in filtrate from bases	0.1404[1]	0.1380	46.80	46.00	46.40
Amino N in filtrate from bases	0.1297	0.1307	43.23	43.57	43.40
Non-amino N in filtrate from bases	0.0107	0.0073	3.56	2.43	2.99
Total N regained	0.3056	0.3025	101.86	100.83	101.34

[1] This result is from a single determination of nitrogen.

13. **Analysis of a 1 per cent hydrochloric acid extract of sphagnum-covered peat and (in part) of calcareous black grass-peat.** Acid extraction was made of the two peats in direct contact with 1 per cent hydrochloric acid. For the extraction 125 gram portions were placed in 2.5 liter acid bottles and two liters of 1 per cent acid added. In the case of calcareous black grass-peat, however, the calculated amount of hydrochloric acid necessary to neutralize the calcium oxide was first added and then sufficient dilute acid to make two liters of a 1 per cent solution. Five hundred

grams were taken in the case of sphagnum-covered peat, while 750 grams were taken in the case of calcareous black grass-peat. The bottles were shaken at intervals during five days and then the contents filtered through two thicknesses of a good grade of cheese cloth and squeezed in the hands. The resulting solution was then filtered through two thicknesses of filter paper on a Buchner funnel.

This extract was colored in each case but the calcareous black grass-peat gave a deeper straw colored solution than did the sphagnum-covered peat. This was probably due to the presence of a larger amount of iron in the one case than in the other. The calcareous black grass-peat is known to contain a very considerable quantity of iron. The wash water in both instances was also straw colored.

It has been shown by a number of investigators, e. g., Jodidi (1909), Kelley and Thompson (1914), and Gortner (1916 a) that considerable amounts of nitrogen are dissolved from certain soils by this preliminary treatment. The acid solution thus obtained should contain the ammonia, acid amides, amines, amino acids, and all other organic nitrogenous compounds soluble in water or very dilute acid. The 1 per cent hydrochloric acid extracted 8.57 per cent of the total nitrogen from sphagnum-covered peat, and 5.09 per cent from the calcareous black grass-peat.

Duplicate nitrogen determinations were made on 250 cc. portions of the acid extract and from these results the total nitrogen in the bulk solution determined. The 5500 cc. solution from sphagnum-covered peat, containing 0.6468 gram nitrogen, and the 5000 cc. from the calcareous black grass-peat, containing 0.4690 gram nitrogen, were used for analysis. These solutions were concentrated under reduced pressure to about 200 cc. and then hydrolyzed for 48 hours, after first adding 75 cc. concentrated hydrochloric acid to the solution from sphagnum-covered peat, and 100 cc. to the solution from the calcareous black grass-peat. During evaporation under reduced pressure considerable hydrolysis took place for the solutions turned dark brown in color. During hydrolysis of calcareous black grass-peat silicic acid separated in the condenser.*

The analysis of sphagnum-covered peat shows that over 65 per cent of the nitrogen is in the form of ammonia. Potter and Snyder (1915 a) have shown that a very small amount of the nitrogen in the 1 per cent hydrochloric acid extract of soils exists in the soil as ammonia nitrogen. It seemed probable that if an extract of the peat contained so much ammonia nitrogen after hydrolysis, the air dry peat must contain an appreciable amount in the ordinary condition. The ammonia nitrogen was determined directly on a 5 gram sample of the air dry material. An excess of calcium hydroxide solution was added and the mixture distilled under reduced pressure for forty-five minutes. It was found that 5.40 per cent of the total

*This was also true with all of the mineral soils studied, and is probably due to the presence of inorganic fluorides.

nitrogen of the soil existed in the form of ammonia nitrogen.*

The precipitates containing the "humin" nitrogen were washed by decantation until practically all the dissolved nitrogen was removed. After digestion the material was diluted to 500 cc. and 250 cc. portions used for distillation. Before concentration the filtrate from the "humin" precipitate of sphagnum-covered peat was reddish in color and after concentration this color changed to a cherry red. Twenty-five grams of phosphotungstic acid was used for precipitation of the bases in sphagnum-covered peat. The barium phosphotungstate precipitate retained 0.0022 gram nitrogen. The solution containing the basic nitrogen was diluted to 50 cc. and the one containing total nitrogen-in-filtrate-from-bases to 250 cc.

The experimental data showing the grams of nitrogen found and per cent of total nitrogen are given in Table XVII.

Table XVII.—Nitrogen distribution of a 1 per cent hydrochloric acid extract of sphagnum-covered peat and (in part) of calcareous black grass-peat.

	Sphagnum-covered peat		Calcareous black grass-peat	
	Grams nitrogen	Per cent of total nitrogen	Grams nitrogen	Per cent of total nitrogen
Total N	0.6468	.....	0.4680	.....
Ammonia N	0.4230	65.40	0.3013	64.38
Humin N	0.0657	10.16	0.0582	12.44
Basic N	0.0389	6.01	.....[1]	.....
Basic N set free as NH_3 by 50% KOH	0.0181	2.80	.....	.....
Basic N not set free as NH_3 by 50% KOH	0.0208	3.21	.....	.....
Amino N of bases	0.0266	4.11	.....	.....
Non-amino N of bases	0.0123	1.90	.....	.....
N in filtrate from bases	0.1335	20.64	.....	.....
Amino N in filtrate from bases	0.1107	17.11	.....	.....
Non-amino N in filtrate from bases.	0.0228	3.53	.....	.....
Total N regained	0.6611	102.21	.....	.....

[1] Distribution of remaining nitrogen not determined.

14. Analysis of a portion of sphagnum-covered peat soluble in 4 per cent sodium hydroxide and precipitated by hydrochloric acid and (in part) of a similar solution from a calcareous black grass-peat. The organic material soluble in 4 per cent sodium hydroxide was next extracted from new portions of the two different peats. Twelve 5 gram portions were leached with 1 per cent of hydrochloric acid to the absence of calcium and the excess of acid removed by washing with distilled water, until the filtrate indicated only a faint trace of free acid when tested with Squibb's litmus paper. After leaching and washing, each 5 gram portion was washed into tall glass-stoppered cylinders of 500 cc. capacity

*This was further indicated by greenhouse experiments. The peat was treated with calcium carbonate at the rate of 4000 pounds per acre and planted to barley. The plants made a very rapid growth during the early stages of development and finally lodged. Next to this was a plot of calcareous black grass-peat which contained only 0.88 per cent of its total nitrogen in the form of ammonia nitrogen. When limed and sown to barley, it did not show any abnormal growth.

with 4 per cent sodium hydroxide and filled to the mark. These were thoroughly shaken and placed on their sides, thus allowing the peat to settle on the sides of the cylinder, thereby exposing a very large surface to the action of the hydroxide. The shaking was repeated at intervals for nine days. The cylinders were then thoroughly shaken, placed in a vertical position and allowed to settle for four days before the supernatant liquid was syphoned off and filtered. The samples of calcareous black grass-peat were almost completely dissolved by the hydroxide solution.

These filtered solutions were neutralized with hydrochloric acid (solution tested faintly acid) when a brown flocculent precipitate separated. This was allowed to settle for several hours and the cider colored solution syphoned off. The brownish black precipitates were filtered and after draining over night were thoroughly mixed with a large volume of water and again filtered and drained. The resulting precipitates were hydrolyzed with 200 cc. of hydrochloric acid for 48 hours. This amount of concentrated acid was added and the flask brought to boiling until hydrogen chloride fumes were evolved showing the presence of constant boiling acid. Silicic acid separated on the condenser during the hydrolysis of calcareous black grass-peat. Even after the hydrolysis there still remained some small lumps of the "humus" precipitate.

The entire hydrolysate was used for the ammonia determination. After this determination the "humin" precipitate was thoroughly ground in a mortar to insure complete disintegration, although this seemed hardly necessary as the solid was already in a fairly fine state of division. This precipitate was washed in the usual manner by decantation, the filtrate concentrated by evaporation and made to 250 cc. volume. Duplicate portions of 25 cc. were used for the determination of total nitrogen in the solution and the result listed as total nitrogen-in-the-filtrate-from-humin. The remaining 200 cc. portion was used for precipitation of the bases and subsequent analysis.

The high content of carbonaceous organic matter made the "humin" precipitate very difficult to digest. The sulfuric acid required was 120 cc. and the digestion extended over 10 days before complete decoloration was effected. Of course precautions were taken to prevent the absorption of ammonia from outside sources. The material was diluted to 500 cc. and 250 cc. portions used in the distillation. The basic nitrogen was precipitated with 25 grams of phosphotungstic acid. The nitrogen retained by the barium phosphotungstate was 0.0043 gram. The solution containing the basic nitrogen was made to 50 cc. volume, and that containing the total nitrogen-in-the-filtrate-from-the-bases was made to a volume of 250 cc.

The experimental data showing the grams of nitrogen and per cent of total nitrogen are given in Table XVIII.

Table XVIII.—Nitrogen distribution in that portion of a sphagnum-covered peat soluble in 4 per cent sodium hydroxide and precipitated by hydrochloric acid and (in part) of a similar solution from a calcareous black grass-peat.

	Sphagnum-covered peat		Calcareous black grass-peat	
	Grams nitrogen	Per cent of total nitrogen	Grams nitrogen	Per cent of total nitrogen
Total N	0.2860	.....	0.6344	.....
Ammonia N	0.0464	16.22	0.0781	12.31
Humin N	0.0950	33.22	0.2093	32.99
Basic N	0.0309	10.80	.....[1]	.....
Basic N set free as NH_3 by 50% KOH	0.0070	2.45	.....	.....
Basic N not set free as NH_3 by 50% KOH	0.0239	8.35	.....	.....
Amino N of bases	0.0174	6.08	.....	.....
Non-amino N of bases	0.0135	4.72	.....	.....
N in filtrate from bases	0.1175	41.08	.....	.....
Amino N in filtrate from bases	0.0965	33.74	.....	.....
Non-amino N in filtrate from bases	0.0210	7.34	.....	.....
Total N regained	0.2898	101.32	.....	.....

[1] Distribution of the remaining nitrogen not determined.

15. Analysis of a portion of sphagnum-covered peat soluble in 4 per cent sodium hydroxide and not precipitated by hydrochloric acid and (in part) of a similar solution from a calcareous black grass-peat. The filtrate remaining from the brownish black precipitate formed by acidifying the sodium hydroxide extracts of the soil with hydrochloric acid (**cf.** section 13) were concentrated in the usual manner to about 700 cc. when a heavy precipitate of sodium chloride separated. On standing over night there also separated a heavy flocculent brown precipitate. This may have been due to the salting out effect of the sodium chloride on some of the organic substances in the solution. The solution was saturated with hydrogen chloride in the cold and the mixture then divided into two portions and hydrolyzed for 48 hours. The cold material after hydrolysis was united and filtered through glass wool and the precipitate washed with concentrated hydrochloric acid. The filtrate was allowed to stand in a tall soil beaker when more salt separated, due to the increased concentration of the hydrochloric acid. The salt that separated was freed from the mother liquid by packing in a centrifuge and washing with acid a number of times. The salt washed as free of the solution as possible was dried on the steam bath. It was nearly white in color. The glass wool was dried and ground with the salt. After being sampled, 15 gram portions were used for Kjeldahl determinations. The results were listed as nitrogen-retained-by-the-salt.

The combined filtrates were concentrated and analyzed. After the digestion of the humin nitrogen, the material in the Kjeldahl flask was made to 500 cc. volume and the distillations carried out on 250 cc. portions. The filtrate and washings from the humin were acidified and evaporated under diminished pressure to less than 200 cc. and then made to 250 cc. volume. Duplicate nitrogen determinations were made on 25 cc. portions of the solution and

the results listed as total nitrogen-in-the-filtrate-from-humin.

The total nitrogen in the hydrolysate was determined by adding the nitrogen obtained as ammonia, and humin (in salt and that precipitated by calcium hydroxide) to the total nitrogen-in-the-filtrate-from-humin.

Fifteen grams of phosphotungstic acid were used for the precipitation of the bases. The barium phosphotungstate from the sphagnum-covered peat retained 0.0055 gram nitrogen. The solution containing the basic nitrogen was made to 50 cc. volume and that containing the total nitrogen-in-the-filtrate-from-the-bases was made to 250 cc. volume.

The experimental data showing the grams of nitrogen and per cent of total nitrogen are given in Table XIX.

Table XIX.—Nitrogen distribution in that portion of a sphagnum-covered peat soluble in 4 per cent sodium hydroxide and not precipitated by hydrochloric acid and (in part) of a similar solution from a calcareous black grass-peat.

	Sphagnum-covered peat		Calcareous black grass-peat	
	Grams nitrogen	Per cent of total nitrogen	Grams nitrogen	Per cent of total nitrogen
Total N	0.3736	.....	0.6204	.....
Ammonia N	0.0993	26.58	0.2003	32.29
Humin N pptd. by $Ca(OH)_2$	0.0335	8.97	0.0652	10.51
Humin N retained in NaCl	0.0102	2.73	0.0169	2.72
Basic N	0.0324	8.67	.....[2]	.....
Basic N set free as NH_3 by 50% KOH	0.0070	1.87	.....	.....
Basic N not set free as NH_3 by 50% KOH	0.0254	6.80	.....	.....
Amino N of bases	0.0160	4.28	.....	.....
Non-amino N of bases	0.0164	4.39	.....	.....
N in filtrate from bases	0.2006[1]	53.69	.....	.....
Amino N in filtrate from bases	0.1721	46.07	.....	.....
Non-amino N in filtrate from bases	0.0285	7.62	.....	.....
Total N regained	0.3760	100.64	.....	.....

[1] Calculated from one determination. Duplicate lost in digestion.
[2] Distribution of the remaining nitrogen not determined.

16. "Jodidi numbers." These determinations were carried out as directed previously on Fargo clay loam, Fargo silt loam, Hempstead silt loam, prairie-covered loess, and forest-covered loess.

The resulting data in grams and in per cent of total nitrogen are shown in Tables XX, XXI, XXII, XXIII, and XXIV. The figures for similar fracions from the complete Van Slyke analyses are added for comparison. These results will be discussed later.

Table XX.—"Jodidi numbers" determined on 100 cc. of hydrolysate of Fargo clay loam, together with a comparison of similar fractions taken from the Van Slyke analysis.

	Grams nitrogen		Per cent of total nitrogen			Average data of Van Slyke analysis Table VIII
	I	II	I	II		Av.
Total N	0.0484	0.0478	.....	.....	.....	.....
Ammonia N	0.0160	0.0160	33.06	33.47	33.26	24.00
Residue from above acidified with HCl and bases pptd. direct, "Basic N".	0.0033	0.0055	6.82	11.51	9.16	9.58
N in filtrate from "bases"	0.0315	0.0277	65.08	57.95	61.52	32.71
Total N regained	0.0508	0.0492	104.96	102.93	103.94	.....

Table XXI.—"Jodidi numbers" determined on 200 cc. of hydrolysate of Fargo silt loam, together with a comparison of similar fractions taken from the Van Slyke analysis.

	Grams nitrogen	Per cent of total nitrogen	Average data of Van Slyke analysis. Table IX.
Total N	0.1424	.....	.....
Ammonia N	0.0487	34.20	26.56
Residue from above acidified with HCl and bases pptd. direct, "Basic N"	0.0193	13.55	12.11
N in filtrate from "bases"	0.0777	54.56	35.14
Total N regained	0.1457	102.31	.....

Table XXII.—"Jodidi numbers" determined on 100 cc. of hydrolysate of Hempstead silt loam, together with a comparison of similar fractions taken from the Van Slyke analysis.

	Grams nitrogen		Per cent of total nitrogen			Average data of Van Slyke ana ysis Table XI
	I	II	I	II	Av.	
Total N	0.0487	0.0513	.....	.....	.....	.....
Ammonia N	0.0207	0.0197	42.51	38.40	40.45	29.44
Residue from above acidified with HCl and bases pptd. direct, "Basic N".	0.0068	0.0067	13.96	13.06	13.51	11.39
N in filtrate from "bases"	0.0243	0.0255	49.90	49.71	49.81	28.10
Total N regained	0.0518	0.0519	106.36	101.17	103.77	.....

Table XXIII.—"Jodidi numbers" determined on 200 cc. of hydrolysate of prairie-covered loess, together with a comparison of similar fractions taken from the Van Slyke analysis.

	Grams nitrogen	Per cent of total nitrogen	Average data of Van Slyke analysis. Table XII.
Total N	0.0601	.....	.....
Ammonia N	0.0244	40.60	30.53
Residue from above acidified with HCl and bases pptd. direct, "Basic N"	0.0036[1]	5.99	12.68
N in filtrate from "bases"	0.0321	53.41	28.80
Total N regained	.....	100.00[2]	.....

[1] By difference. [2] Calculated.

Table XXIV.—"Jodidi numbers" determined on 200 cc. of hydrolysate of forest-covered loess, together with a comparison of similar fractions taken from the Van Slyke analysis.

	Grams nitrogen	Per cent of total nitrogen	Average data of Van Slyke analysis. Table XIII.
Total N	0.0340	.....	.....
Ammonia N	0.0130	38.24	28.69
Residue from above acidified with HCl and bases pptd. direct, "Basic N"	0.0059	17.35	13.98
N in filtrate from "bases"	0.0175	51.47	27.21
Total N regained	0.0364	107.06	.....

17. **Summary Tables.** Certain of the preceding analyses have been summarized in Tables XXV, XXVI. In Table XXV are shown the amounts and percentages of soil dissolved by the acid during hydrolysis as well as the amount of nitrogen and percentage of the total nitrogen dissolved. Table XXVI summarizes average nitrogen distribution of Tables V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XVI, XVII, XVIII, and XIX.

Table XXV.—Percentages of soil and of soil nitrogen dissolved by hydrolyzing the different soil types.

Soil type	Sample	Grams soil taken (dry basis)	Grams soil dissolved	Per cent soil dissolved	Grams nitrogen in soil	Grams nitrogen dissolved[1]	Per cent of dissolved nitrogen
Fargo clay loam	I	240.2	43.2	17.99	0.6005	0.4252	70.79
	II	240.2	43.2	.....	.....	.....	.....
Fargo silt loam	I	222.8	54.8	24.60	1.8336	1.4237	77.65
Carrington silt loam	I	235.5	36.5	16.61	0.8738	0.6191	70.91
Hempstead silt loam	I	242.3	32.3	13.33	0.6201	0.4395	70.87
	II	242.3	33.3	13.74	.....	0.4508	72.69
Prairie-covered loess	I	230.3	42.3	18.37	0.6933	0.5260	75.91
	II	230.3	45.3	19.64	.....	0.4991	72.02
Forest-covered loess	I	294.4	29.4	9.98	0.3768	0.2735	72.19
	II	294.4	32.4	11.11	.....	0.2511	66.65

[1] The figures in this column were obtained by subtracting the "insoluble humin nitrogen" remaining in the residual soil from the nitrogen figures obtained by multiplying the original weight of soil taken (dry basis) by the nitrogen content of the soil. These figures may or may not agree with the figures obtained in kjeldahling a portion of the solution, due to experimental errors, and perhaps to errors introduced in using a uniform factor (2.6) for specific gravity. The figures in this column are free from any error of this sort.

Table XXVI.—Average nitrogen distribution in per cent of the total nitrogen as given in preceding Tables.

	Sphagnum-covered peat.	Sphagnum-covered peat + subsoil.	Sphagnum-covered peat + metallic tin.	Sphagnum-covered peat soluble in 1% HCl extract.	Sphagnum-covered peat soluble in 4% NaOH not pptd. by HCl.	Sphagnum-covered peat soluble in 4% NaOH pptd. by HCl.	Calcareous black grass-peat.	Acid "muck" soil.	Fargo clay loam.	Fargo silt loam.	Carrington silt loam Sample I.	Carrington silt loam Sample II.	Hempstead silt loam.	Prairie-covered loess.	Forest-covered loess.
	Table VI	Table XIV	Table XVI	Table XVII	Table XIX	Table XVIII	Table V	Table VII	Table VIII	Table IX	Table X	Table X	Table XI	Table XIII	Table XII
Ammonia N	23.27	25.52	20.18.	65.40	26.58	16.22	19.26	19.49	24.00	26.56	28.55	28.07	29.44	30.53	28.69
Insoluble humin N in soil	*	*	*	*	2.73	*	*	*	28.27	22.93	25.84	24.59	27.56	24.35	26.92
Humin N pptd. by $Ca(OH)_2$	*	*	*	*	8.97	*	*	*	9.21	[1]3.26	5.93	6.63	4.80	5.19	4.84
Total humin N	26.38	26.96	22.48	10.16	11.70	33.22	26.07	27.61	37.48	26.19	31.77	31.22	32.36	29.54	31.76
Basic N	9.73	10.98	12.28	6.01	8.67	10.80	10.60	13.55	9.58	12.11	11.71	14.74	11.39	12.68	13.98
Basic N set free as NH_3 by 50% KOH	2.98	4.09	3.31	2.80	1.87	2.45	3.27	3.10	3.23	3.20	3.36	3.17	2.28	2.92	3.69
Basic N not set free as NH_3 by 50% KOH	6.75	6.89	8.97	3.21	6.80	8.35	7.33	10.45	6.35	8.91	8.35	11.57	9.10	9.76	10.29
Amino N of bases	5.26	7.29	6.86	4.11	4.28	6.08	6.35	9.13	6.44	7.52	8.04	7.99	7.44	7.48	7.57
Non-amino N of bases	4.47	3.69	5.41	1.90	4.39	4.72	4.25	4.42	3.14	4.59	3.67	6.75	3.95	5.20	6.41
N in filtrate from bases	43.00	38.96	46.40	20.64	53.69	41.08	42.40	38.81	32.71	35.14	25.47	28.25	28.10	28.80	27.21
Amino N in filtrate from bases	40.45	33.82	43.40	17.11	46.07	33.74	39.46	33.82	30.77	32. 64	**	25.85	24.56	26.24	25.17
Non-amino N in filtrate from bases	2.55	5.14	2.99	3.53	7.62	7.34	2.94	4.99	1.95	2.50	.....	2.40	3.54	2.56	2.04
Total N regained	102.28	102.42	101.34	102.21	100.64	101.32	98.33	99.46	103.77	[2]100.00	97.50	102.28	101.29	101.56	101.64

*Not determined separately.
**Solution lost at this point.
[1] By difference.
[2] Calculated.

18. An attempt to isolate pure proteins from a soil. From all the positive evidence it would seem that a very considerable portion of the nitrogen in the soil exists in other forms than protein. For example, Potter and Snyder (1916) found an average of 20.46 per cent of the alkali soluble nitrogen of a soil to be non-protein in nature. Bacteria and fungus spores all contain chitin which on hydrolysis yields glucosamine which will give amino ($-NH_2$) nitrogen of non-protein origin.

The average C/N ratio that exists in this series of six mineral soils studied has been shown by Gortner (1916 a) to be 12.23. The average nitrogen content of the six soils is 0.355 per cent which would indicate 2.22 per cent protein (N×6.25) if all of the nitrogen existed in this form. However, the total organic matter in these soils averaged 7.48 per cent (carbon×1.724), showing that less than 30 per cent of the soil organic matter could be of protein nature. This is shown equally well by a comparison of the C/N ratios. The average analyses of sixteen plant proteins as recorded by Mathews (1915) gives carbon 52.08 per cent and nitrogen 17.73 per cent. The average C/N in these vegetable proteins is 2.94. The high C/N ratio in soils indicates that the organic matter does not consist essentially of protein material (**cf.** however, Gortner 1917, when a ratio of 3.06 was found indicating that the organic matter in this instance was essentially protein).

In view of the desirability of demonstrating the presence or absence of proteins in soils an attempt was made to isolate from a soil either alcohol soluble or salt soluble proteins. A 6 inch bulk sample of Fargo silt loam from Morristown, Rice County, containing 0.397 per cent of nitrogen in the air dry soil, was used.

a. Extraction with 70 per cent ethyl alcohol. The soil was first leached with 1 per cent hydrochloric acid to the absence of calcium and washed with distilled water until practically all chlorides were removed. For this purpose sixty-one 100 gram portions were taken. It required about 150 liters of acid to remove all traces of calcium.

After leaching, the soil was allowed to air dry in the greenhouse and there remained 5700 grams air dry soil. Five hundred gram portions were placed in twelve 2.5 liter acid bottles and extracted successively with 70 per cent ethyl alcohol. A fresh portion of alcohol was added to the first bottle of the series, shaken, allowed to stand over night, syphoned off, and placed in the bottle next ahead in the series. This was continued until six successive portions of fresh alcohol had been added. The alcohol was absorbed by the dry soil to such an extent that a 500 cc. portion of fresh alcohol had to be added to the bottles towards the end of the series. Almost 15 liters of alcohol were used, but only a little over 10 liters were regained at the end, since so much was retained by the soil.

The combined extracts were filtered until practically free of clay and then concentrated under diminished pressure at a temperature of 55° C. The extracts were in all cases straw colored.

A heavy yellow precipitate separated on concentration but dissolved very largely in the fresh portions of the alcohol extract The greater portion of water was finally removed by evaporating four times with 93 per cent alcohol. More water, however, could have been removed by evaporating with absolute alcohol. The trace of clay, remaining after concentration, was filtered off on a dry filter paper and the solution diluted to 500 cc. with 93 per cent alcohol. The total nitrogen was determined on duplicate 25 cc. portions of the solution by the Kjeldahl method. **The results gave 0.1320 gram nitrogen in the total alcohol extract.**

Tests were made on portions of this alcohol extract for the presence of proteins or protein-like substances. Precipitates were obtained with phosphotungstic acid and lead acetate. A heavy yellow precipitate formed on dilution with water. This yellow precipitate was readily soluble in sodium hydroxide (suggests presence of acids or phenols), and in concentrated hydrochloric acid. **The biuret test and Liebermann's reaction both gave negative tests, and Millon's reaction gave an extremely faint test (mere trace).**

One 25 cc. portion of the alcohol solution was diluted with water and extracted four successive times with chloroform, followed by a single extraction with ether. The chloroform and ether extracts were combined and evaporated to dryness in a platinum dish on the steam bath and later in a hot air oven at 102° C. and the residue weighed. The results indicated the presence of 0.3108 gram of organic matter, making a total of 6.2160 grams in the entire alcohol extract.

After the chloroform-ether extraction there remained a dark brown granular insoluble substance. This was filtered off on a 5 cm. Buchner funnel and the filter and precipitate used for the determination of nitrogen. The results indicated the presence of 0.0010 gram **maximum** protein nitrogen in this solution, leaving 0.1310 gram of non-protein nitrogen in the same extract.

The negative color tests and the small amount of nitrogen indicate that no protein is present in this soil that is soluble in alcohol.

Another 25 cc. portion of the alcohol extract was evaporated to dryness on the water-bath in a platinum dish and subsequently dried in an oven at 96° C. with a short final heating at 103° C. The organic matter amounted to 0.6126 gram, making a total content of organic matter in the alcohol solution 12.2520 grams. From the data given it was found that only 1.08 per cent of the organic matter in the 70 per cent alcohol extract of the soil was nitrogen.

b. Extraction with absolute alcohol. A 100 gram portion of the unleached soil was extracted with absolute alcohol for 60 hours in a Soxhlet extraction apparatus. The extraction flask had a capacity of 500 cc. and all the joints of the apparatus were ground glass. Several pieces of broken porcelain were placed in the flask to prevent bumping, as the amount of dissolved organic matter increased. An alundum extraction thimble was used to hold the

soil. The alcohol syphoning back became colorless several hours before the extraction was stopped. At the end of the extraction the solution in the flask was deep straw color.

The total alcohol extract was evaporated to small volume and then the total nitrogen determined, which was found to be 0.0011 gram. This was equivalent to 0.0660 gram of nitrogen in 6 kilo of soil before leaching with acid. It will be recalled that 0.1320 gram nitrogen dissolved in the 70 per cent alcohol after first leaching with 1 per cent hydrochloric acid.

This would indicate that many of the organic nitrogenous compounds are in combination with the lime or other bases present in the soil, and that this combination is broken up when leached with 1 per cent hydrochloric acid.

c. Extraction with 10 per cent sodium chloride. After completion of the alcoholic extraction, the soil residue was dried in an air oven at about 65° C. and placed in a 20 liter bottle. To this was added 2000 cc. of 10 per cent sodium chloride solution for each kilo of dry soil. The bottle was shaken repeatedly and allowed to settle over night. The presence of an electrolyte caused the clay to settle rapidly, so that a clear solution could be withdrawn. After the soil had been in contact with the salt solution 20 hours, two 100 cc. portions were withdrawn and analyzed for nitrogen. The results showed that 0.1350 gram nitrogen was contained in 5 liters of the supernatant liquid. After shaking and standing for three days duplicate determinations were again made on 100 cc. portions with the result that 0.1600 gram nitrogen was present in the corresponding solution. This indicates that the longer the soil is in contact with the sodium chloride solution the greater the amount of organic nitrogen extracted.

Two 500 cc. portions of the salt extract were used for precipitation with phosphotungstic acid. After the addition of 45 cc. of concentrated hydrochloric acid, the phosphotungstic acid was added. The gelatinous precipitates formed were filtered on an ordinary funnel and washed with the filtrate, and then used for the determination of nitrogen by the usual method. The duplicates averaged 0.0028 gram nitrogen, thus making the total nitrogen precipitated by phosphotungstic acid 0.0280 gram in the 5 liters of supernatant liquid. This shows that 17.50 per cent of the nitrogen extracted from the residual soil was precipitated by phosphotungstic acid. This represented the maximum protein nitrogen in the 10 per cent sodium chloride solution.

III. DISCUSSION.

A. Changes in nitrogen distribution in a protein when hydrolyzed in the presence of a mineral soil. From a study of Table III it is seen that the histidine nitrogen formed when fibrin is hydrolyzed alone is 4.36 per cent and when hydrolyzed in the presence of cellulose it is 4.86 per cent of the total nitrogen. By referring to Table II where fibrin is hydrolyzed in the presence of ignited subsoil we see the histidine nitrogen is entirely lacking and that the nitrogen precipitated by calcium hydroxide amounts to 4.81 per cent. This corresponds very closely to the amount of histidine found in the other two cases. In other points the three analyses agree within experimental error.

It appeared possible that the histidine nitrogen might have been converted into the nitrogen fraction precipitated by calcium hydroxide. It is a well known fact that histidine can be precipitated by silver nitrate in slightly alkaline solutions. Since there are a large number of mineral constituents in the soil it may be possible that the histidine could be precipitated by some of these and thus be found with the calcium hydroxide precipitate.

With this idea in mind an analysis of histidine was made in the presence of an ignited subsoil, only three fractions being determined. One 0.5000 gram sample of histidine di-hydrochloride* and 50 grams ignited subsoil were boiled in the presence of 100 cc. of hydrochloric acid (sp. gr. 1.18) for 48 hours. The solution was diluted to 500 cc. in a graduated flask and two 200 cc. portions syphoned off and analyzed. The solution was deep straw color. The determinations for ammonia nitrogen gave negative results. The precipitate formed by calcium hydroxide was washed by decantation until free of dissolved nitrogen compounds, and the total nitrogen determined. This precipitate was bulky due to the presence of large amounts of ferric and aluminum hydroxides. The average humin nitrogen in the two samples was only 0.0006 gram.

The filtrate from the calcium hydroxide precipitate was concentrated to a small volume and the entire solution used for the nitrogen determination. Sample I, contained 0.0371 gram in the filtrate, and Sample II, 0.0365 gram, making an average of 0.0368 gram.

The residual soil was practically colorless, and a determination indicated that it was nitrogen free. The volume occupied by the soil residue was 17.3 cc. By calculation it was found that the total nitrogen regained in the original solution was 0.0903 gram, theoretical 0.0921 gram, or a recovery of 98.01 per cent.

Thus practically all of the histidine was recovered in the filtrate from the calcium hydroxide precipitate, indicating that the hypothesis was incorrect.

*The histidine di-hydrochloride was prepared from dried blood as outlined by Abderhalden (1910). Total nitrogen found 18.42 per cent; calculated 18.42 per cent.

It is possible that the specific character of the histidine nitrogen is destroyed by certain oxidizing agents present during the hydrolysis, with the result that histidine nitrogen is not precipitated in the basic fraction. A study is being conducted at the present time on the hydrolysis of pure protein in the presence of certain inorganic oxidizing agents. It is hoped that some light will be thrown upon the disappearance of histidine as well as the formation of "humin" and of the "nitrogen precipated by calcium hydroxide."

It is of interest to note that the average total nitrogen in the two samples in Table II is 0.4584 gram (cf. Gortner 1916 c, where total nitrogen on four determinations of three grams averaged 0.4551), showing that all of the nitrogen present is accounted for in the method of analyses used.

Table IV shows the difference between the duplicate determinations of the analysis of fibrin alone and in the presence of carbohydrate and of subsoil, and the differences apparently due to the addition of 100 grams ignited subsoil to the 3 grams of fibrin. Van Slyke's (1911) "maximum" and "average" differences to be expected between duplicate determinations are also given in the table for reference.

From a study of Table IV it will be seen that the difference between the analyses of fibrin hydrolyzed alone and in the presence of ignited subsoil are, in the case of most of the fractions, within the maximum allowed by Van Slyke for experimental error. The only differences which are certainly greater than experimental error are those of humin nitrogen, histidine nitrogen, and amino nitrogen in the filtrate from the bases. It is observed that practically the same error occurred with the amino nitrogen in the filtrate from the bases when hydrolysis was carried out in the presence of carbohydrate.

From this analysis one can only draw the conclusion that even if **the organic matter of the soil consisted entirely of pure protein, one would not obtain the same nitrogen distribution by the Van Slyke analysis in the presence of soil that one would obtain in the absence of the soil,** or in other words, the presence of ignited mineral subsoil interferes with the Van Slyke analysis in much the same manner as carbohydrates (Gortner 1916 c, and Hart and Sure 1916).

B. The humin nitrogen, its origin and significance. In such a discussion one must first consider the source of humin nitrogen in pure proteins.

Osborne and Jones (1910) suggest that perhaps tryptophane and histidine are responsible for the humin formation, basing their postulation on the fact that zein, which contains no tryptophane and but little histidine, gives only small amounts of humin on hydrolysis.

Gortner and Blish (1915) hydrolyzed zein in the presence of both tryptophane and of histidine and found that a large part of the tryptophane was converted into humin nitrogen, whereas none

of the histidine was converted into humin but was all recoverable in the bases. I have shown that histidine is practically all recovered in the filtrate from the humin when it is hydrolyzed in the presence of an ignited mineral subsoil. Histidine, therefore, can be eliminated as a factor in the formation of humin nitrogen in the soil. Gortner and Blish conclude that—

> in all probability the humin nitrogen of protein hydrolysis has its origin in the tryptophane nucleus.

Gortner (1916 c) has shown that the humin nitrogen is increased by the addition of carbohydrate material to protein, and suggests that this increase may be due to both physical and chemical causes.* He presents evidence to show that the action of carbohydrate is probably due to the furfural produced from the carbohydrate and shows that increasing quantities of furfural cause the humin nitrogen to steadily increase, and the work of Gortner and Holm (1917) shows that the presence of formaldehyde during hydrolysis causes a gradual increase in the amount of humin nitrogen up to a maximum very much larger than the amount of normal humin nitrogen, and then a decrease with increased amounts of aldehyde.

Shmook (1914) states that during the hydrolysis of his soils there separated on the walls of the condenser a substance violet blue in color, and that this appears during the hydrolysis of pure protein and is recognized as Liebermann's reaction for protein substances. The above conclusion in regard to the hydrolysis of a pure protein is incorrect, since no color appears on the neck of the flask or condenser in such an analysis. When furfural is heated alone with hydrochloric acid a characteristic colored substance is deposited on the condenser. It has been shown by Gortner (1916 c) that at the same time a polymerization (?) of furfural to humin takes place very rapidly. He found that when 1.165 grams of furfural were heated with 100 cc. of hydrochloric acid (sp. gr. 1.115) for 18 hours that 76.40 per cent of the original furfural was converted into insoluble "humin." Our mineral soils on hydrolysis gave a deposit on the condenser similar to that described by Shmook. The reaction indicates the presence of furfural, which is in turn formed from the carbohydrates in the soil. This must be considered to be a distinctive furfural reaction.

The humin nitrogen actually present in the soil may easily be a very small part of the nitrogen found. It is evident that there must be many nitrogenous organic compounds present in the soil which have no relation to protein material, such as purine, pyrimidine bases, nitrogenous lipins, and nitrogenous pigments besides a number of other non-protein substances. It is certain that the humin nitrogen will be greatly changed by the presence of many of these compounds. The calcium hydroxide here drags down all the organic nitrogenous compounds which are soluble in dilute acids, but insoluble in hot water and dilute calcium hydroxide, to-

*Practically the same increase in humin nitrogen occured when fibrin was hydrolyzed in the presence of a mineral subsoil. The humin in this case was not due to the presence of carbohydrate since the soil had lost all of its organic matter by ignition.

gether with the calcium salts of nitrogenous organic acids, the calcium salts of the purine and pyrimidine bases in addition to the humin formed from the protein material, and other organic compounds that are adsorbed, absorbed, occluded, or combined with the iron and aluminum hydroxides present.

From Table XXVI we find that from 4.84 to 9.21 per cent of the total nitrogen is precipitated by calcium hydroxide. It can readily be seen that this does not represent true humin nitrogen, since the calcium hydroxide does not contain any black colored substances formed by hydrolysis. The solution from which it is precipitated is colored only by ferric compounds, therefore, the organic material in this precipitate must consist of colorless organic compounds adsorbed by or combined with the lime. This portion of the nitrogen consists almost certainly of non-protein material. In all pure proteins the nitrogen retained in the calcium hydroxide precipitate is supposed to consist entirely of deeply colored compounds. This study of the distribution of organic nitrogen in the soil has led to a new fraction, not previously reported. Certain of the analyses were carried out before the importance of this fraction was realized, but in most of the analyses I have reported this fraction as "nitrogen precipitated by calcium hydroxide," because of its unknown nature. Further investigations of this fraction are highly desirable.

Another point of interest is observed in the humin nitrogen of the sphagnum-covered peat hydrolyzed in the presence of metallic tin. There is a decided decrease in this fraction as compared with the peat hydrolyzed alone. As noted earlier, Samuely (1902), suggested that humin formation might be due to an oxidation process and certain of the earlier workers (**cf.** Hlasiwetz and Habermann 1871 and 1873) hydrolyzed protein in the presence of stannous chloride in order to obtain a colorless solution instead of one deeply colored by the presence of humin.

I, therefore, hydrolyzed some gliadin in the presence of tin and found that while the solution remained colorless, nevertheless small balls of **black** material were formed. The humin nitrogen (insoluble in acid + that pptd. by $Ca(OH)_2$) was 1.17 per cent of the total nitrogen, while gliadin hydrolyzed alone gave a dark colored hydrolysate and a humin nitrogen content of only 0.67 per cent.

Recently Spriestersbach (private communication) has hydrolyzed fibrin (from a different sample than mine) alone and in the presence of stannous chloride and finds in the fibrin hydrolyzed alone 1.67 per cent of total humin nitrogen, of which 1.06 per cent is "acid insoluble" (**cf.** Gortner 1916 c) and 0.61 per cent precipitated by calcium hydroxide. In the sample of fibrin hydrolyzed in the presence of tin he finds 0.91 per cent of total humin nitrogen, of which 0.25 per cent is "acid insoluble" and 0.66 per cent precipitated by calcium hydroxide. The nature of his hydrolysate agreed in all respects with mine, i.e., was colorless or faint straw color, with tiny black balls of humin floating on the surface or at-

tached to the sides of the flask in which the hydrolysis was carried out.

These experiments suggest interesting possibilities, but discussion must be deferred until we know more of the exact reactions taking place.

The **true humin nitrogen** remains in the residual soil after hydrolysis. The amount of nitrogen in this fraction varies from 22.93 per cent to 28.27 per cent of the total nitrogen for the mineral soils studied. This represents more nearly the true humin nitrogen, in that the black coloring matter formed by acid hydrolysis remains in this portion, but in addition we should also find here all organic nitrogenous compounds insoluble in fairly strong solution of hydrochloric acid, all of the nitrogen adsorbed by the carbohydrate humins, etc. Potter and Snyder (1915 a) express surprise at the large proportion of nitrogen in this fraction but when one considers the heterogeneous nature of the soil organic matter it is perhaps **more surprising** to find that over 60 per cent of the nitrogenous compounds are soluble in strong hydrochloric acid. Further study is necessary before the full significance and origin of this humin nitrogen can be thoroughly understood.

C. The effect of the quantity of acid used for the hydrolysis on the amount of nitrogen dissolved and the nitrogen distribution in soils. Throughout this investigation acid at least as strong as constant boiling hydrochloric acid was used for the hydrolysis since that is the strength recommended for the analysis of pure proteins.

In the case of two soils, however, one of the duplicates was hydrolyzed in the presence of 1000 cc. concentrated acid to 250 grams of soil, the other being hydrolyzed in the presence of 500 cc. of constant boiling acid to 250 grams of soil, in order to see if any noticeable differences would be observed on the resulting analyses. The two soils thus hydrolyzed were the prairie-covered loess and forest-covered loess.

The results show little difference between the duplicates. Table XXV shows that the larger volume of the stronger acid dissolved a greater per cent of the soil, due to the fact that more of the mineral constituents were soluble in acid of this concentration. At the same time, however, the amount of nitrogen extracted was less.

D. The percentage of soil nitrogen extracted by acid hydrolysis. Shorey (1905) working with a single Hawaiian soil extracted 84.68 per cent of the total soil nitrogen by acid hydrolysis. Jodidi (1911) working with eleven Iowa soils found from his studies a minimum of 68.90 per cent, a maximum of 83.94 per cent, and an average of 75.77 per cent; Lathrop and Brown (1911) in five Pennsylvania soils found a minimum of 70.60 per cent, a maximum of 73.71 per cent, and an average of 71.78 per cent; Shmook (1914), working with four Russian soils, found a minimum of 60.60 per cent in the Laterite soil, a maximum of 87.67 per cent in the Podzol soil, and an average of 68.33 per cent; Kelley (1914),

working with nine soils of the Laterite class common to the Hawaiian Islands, found a minimum of 67.51 per cent, a maximum of 91.80 per cent, and an average of 82.17 per cent; and Potter and Snyder (1915 a) in seven Iowa soils, found a minimum of 68.68 per cent, a maximum of 76.47 per cent, and an average of 74.41 per cent.

The grand average of all of these thirty-seven soils from widely different origin gives 75.91 per cent of the soil nitrogen in solution in the hydrochloric acid extract. In my studies I found a minimum of 66.63 per cent, a maximum of 77.65 per cent, and an average of 72.19 per cent extracted by the acid.

These results indicate that the nitrogen of practically all soils, in so far as investigated, dissolves to about the same extent during the acid hydrolysis.

E. "Jodidi numbers." A study of Tables XX, XXI, XXII, XXIII, and XXIV shows that the nitrogen distribution by this method does not give accurate results when compared with similar fractions of the Van Slyke analyses. The ammonia nitrogen and nitrogen in the filtrate from "bases" are all much too high, while "basic N" corresponds fairly well with the true basic nitrogen. If one desires accurate data in regard to the distribution of the organic nitrogen in the soil he should not use "Jodidi numbers."

F. Attempts to extract proteins from the soil. The attempt to isolate alcohol or salt soluble proteins from the soil was not successful. The **maximum** protein nitrogen in the 70 per cent alcohol extract from 6 kilo of soil amounted to only 0.0010 gram, while the **maximum** protein nitrogen extracted by the 10 per cent sodium chloride amounted to 0.0280 gram or 17.50 per cent of the total nitrogen in solution. A larger amount of organic nitrogen was extracted by alcohol when the soil was first leached with 1 per cent hydrochloric acid.

The amounts of possible protein were so small that it seems safe to conclude that no appreciable quantities of alcohol soluble or salt soluble proteins are found in the soil.

G. A consideration of the nitrogen distribution in different extracts from the sphagnum-covered peat. Nitrogen distribution was determined on extracts of a sphagnum-covered peat soluble in (a) 1 per cent hydrochloric acid, (b) 4 per cent sodium hydroxide and **not** precipitated by acidification, and (c) 4 per cent sodium hydroxide and precipitated by acidification with hydrochloric acid. Of the three extracts only the second approximates the distribution of nitrogen in a pure protein. The figures for the ammonia nitrogen are abnormally high in the hydrochloric acid extract.

The humin nitrogen is high in all the extracts, but is excessive in fraction (c). It is clear that carbohydrates from the soil must be present in all three fractions used, and must have some share in bringing the humin nitrogen up to such high figures. The nucleic acids (Shorey 1911a, 1912) would be found in the hydrochloric acid precipitate from the sodium hydroxide solution, and

the purine and pyrimidine compounds of these nucleic acids, as well as the lecithins (Aso 1904, Stoklasa 1911) and nitrogenous lipins and nitrogenous acids would be precipitated with the true humin by the calcium hydroxide.

The basic nitrogen figures are not widely divergent although there may be some significant differences. The differences between the nitrogen in the filtrate from the bases is perhaps the most significant of all. An amino nitrogen of only 17.11 per cent in the filtrate from the bases such as is found in the hydrochloric acid extract is far lower than has ever been obtained in an analysis of a pure protein and indicates that the nitrogen of this extract is essentially non-protein.

Unfortunately it was impossible to complete the corresponding analyses on the calcareous black grass-peat, but the fractions obtained would indicate a distribution similar to that of the sphagnum-covered peat.

H. General conclusions in regard to the distribution of soil nitrogen in different soil types. From a study of Table XXVI we observe a great similarity between the different fractions. This is practically the same deduction made by Potter and Snyder (1915 a) in regard to their study made on a single soil type under different fertilizer treatment.

I find that the nitrogen distribution in a soil is very uniform whether in the same soil type under different fertilizer treatment, or in different soil types. This is to be expected, for if one were to take fifty Van Slyke analyses of protein at random and compare the average analyses with that of another fifty analyses, one should expect to find results agreeing closely with each other. This expectation should also hold true for the hydrolysate of soils, since in each soil are to be found many of the nitrogenous compounds contained in the plant and animal products that find their way to the soil together with their decomposition products. Since there is such a great variety of different nitrogenous substances in the soil, it stands to reason that the nitrogen distribution in soils is an **average** distribution, and as such should not be expected to vary widely from soil to soil.

It has been shown in the earlier part of this discussion that when fibrin was hydrolyzed in the presence of ignited subsoil, no histidine fraction was obtained.

For reasons which were stated previously, I have not tabulated the nitrogen distribution under the different headings used for the analysis of pure proteins. However, in view of the results obtained on the fibrin hydrolyzed in the presence of ignited subsoil, it is perhaps worth while to consider what values the histidine fraction would have had. The Fargo clay loam, Fargo silt loam, and Sample I of the Carrington silt loam, gave results indicating that this fraction was absent, while Sample II of Carrington silt loam gave 2.97 per cent, Hempstead silt loam 0.78 per cent, prairie-covered loess 1.21 per cent, and forest-covered loess 1.25 per cent of histidine nitrogen.

Potter and Snyder (1915 a) find this fraction to be present in all of their eight soils, with a minimum of 1.99 per cent and a maximum of 6.30 per cent. They do not give sufficient analytical data to permit a recalculation of their figures in order to ascertain if any errors in calculation were made. However, it is possible that their finding is due to the fact that all of their mineral soils represented a single soil type. It is possible that this form of nitrogen may have been especially abundant in their particular soil. It is of particular interest to note that their soil was from the Wisconsin drift area, as was my sample of Carrington silt loam from Morristown, which gave my maximum amount of nitrogen in this fraction. The sample of Carrington silt loam from Nerstrand was situated on the Kansan drift and gave no "histidine" nitrogen.

IV. SUMMARY.

This paper deals with a study of the nitrogen distribution in different soil types, by applying Van Slyke's method. Tables have been presented showing such distribution for the following materials:

a. Fibrin hydrolyzed in the presence of an ignited mineral subsoil (together with data of fibrin hydrolyzed alone and in the presence of carbohydrates).

b. A calcareous black grass-peat.

c. An acid, sphagnum-covered peat, hydrolyzed alone, in the presence of a mineral subsoil, and in the presence of stannous chloride.

d. An acid "muck" soil.

e. Seven samples of mineral surface soil representing the following soil types: Fargo clay loam, Fargo silt loam, Carrington silt loam (two samples from different glacial drifts), Hempstead silt loam, prairie-covered loess, and forest-covered loess.

f. Extracts of a sphagnum-covered peat and of a calcareous black grass-peat soluble in (a) 1 per cent hydrochloric acid, (b) 4 per cent sodium hydroxide but precipitated by acid, and (c) 4 per cent sodium hydroxide and not precipitated by hydrochloric acid.

The following conclusions are evident:

1. The figures for the ammonia nitrogen in a protein analysis are not appreciably changed when the hydrolysis is carried out in the presence of an ignited mineral soil equal to twenty times the weight of the protein material.

2. The "humin" nitrogen is greatly increased by the addition of ignited mineral soil. It was shown that histidine nitrogen cannot account for this increase, neither is it due to the presence of carbohydrates, since the soil lost all its organic matter on ignition.

3. Attention has been called to the fact that the analysis of a pure protein in the presence of even an ignited mineral soil does not give reliable results for the different fractions, and that such determinations are of value only when used for purposes of comparison. Such data should not be compared with analyses of pure proteins.

4. Since practically all mineral soils give furfural on treatment with acid it is very likely that a very considerable amount of the total humin nitrogen found is due to the presence of carbohydrates in the soil, which give rise to furfural during hydrolysis. This may combine with certain of the nitrogenous compounds and cause an increase in the "humin" nitrogen, as well as adsorb or occlude nitrogenous compounds in the "humin" formed from furfural by polymerization.

5. This investigation of the distribution of organic nitrogen in the soil indicates a new fraction, the nature of which has not been previously recognized. This is the fraction of nitrogen removed from a colorless solution by calcium, iron, and aluminum hydroxides on the addition of calcium hydroxide. The nitrogen retained in this fraction must consist almost entirely of non-protein material, since the organic substances in this precipitate have been shown to be **colorless** organic compounds adsorbed by or combined with the metallic hydroxides. This fraction has been reported as nitrogen precipitated by calcium hydroxide.

6. The **true** humin nitrogen remains in the residual soil after hydrolysis, but in addition non-humin nitrogenous compounds must also be retained in this fraction.

7. The strength and volume of the hydrochloric acid used in hydrolysis has little effect on the nitrogen distribution of the hydrolysate provided acid as strong as constant boiling acid is used, in the proportion of at least two parts of acid to one of soil.

8. Results gained from a study of different soils indicate that the organic nitrogen dissolves, during hydrolysis, to almost the same extent regardless of the origin and nature of the soil.

9. Some very interesting figures are found in the comparison of the different extracts from sphagnum-covered peat (Table XXVI). The portion soluble in sodium hydroxide and **not** precipitated by hydrochloric acid gives a nitrogen distribution approximating very closely that of a normal plant protein. The nitrogen dissolving in the preliminary hydrochloric acid leaching shows a nitrogen distribution which is **certainly** not due exclusively to protein materials, **e. g.,** an ammonia nitrogen percentage of 65.40 and amino-nitrogen-in-filtrate-from-bases of 17.11 per cent.

10. When an attempt was made to isolate alcohol soluble and salt soluble proteins from the soil the amounts obtained were so small that it seems safe to conclude that no appreciable quantities of these types of proteins are present.

11. The most significant fact brought out by this study is that the organic nitrogen distribution in **different soil types** is very uniform. This is to be expected since it has been pointed out that the nitrogen distribution in soils is an **average** distribution of all the plant and animal nitrogenous products that find their way to the soil.

V. LITERATURE CITED

ABDERHALDEN, EMIL.
1910. Handbuck der Biochemischen Arbeitsmethoden, Bd. II, 1101 p. Urban and Schwarzenberg, Berlin and Wien.

ASO, K.
1904. On organic compounds of phosphoric acid in the soil. *Bull. Coll. Agr. Tokyo,* v. 6, pp. 277-284.

BAUMANN, A.
1887. Ueber die Bestimmung des im Boden enthaltenen Ammoniak-Stickstoffs und über die Menge des assimilierbaren Stickstoffs im unbearbeiteten Boden. *Landw. Vers. Stat.,* Bd. 33, pp. 247-303.

BERTHELOT AND ANDRE.
1886. Sur les principes azotés de la terre végétale. *Compt. rend.,* t. 103, pp. 1101-1104.

BERZELIUS JÖNS JACOB.
1808-18. Lärbok i Kemien, Upsala. 3 vols.

BERZELIUS, J. J.
1838. Untersuchung einiger Säuren, welche mit Schwefelsäure aus organischen Stoffen gebildet werden. *Poggendorff's Ann. Phys. u. Chem.,* Bd. 44, pp. 369-414.

BOCKLISCH, O.
1885. Ueber Fäulnissbasen (Ptomaïne) aus Fischen. *Ber.,* Bd. 18, pp. 87-89.

BOUSSINGAULT, J. B.
1838. Recherches chimiques sur la Végétation, entreprises dans le but d'examiner si les Plantes prennent de l'Azote à l'atmosphère. *Ann. chim. phys.,* t. 67, pp. 5-54.

BOUSSINGAULT, J. B.
1838a. Recherches chimiques sur le Végétation, entreprises dans le but d'examiner si les Plantes prennent de l'Azote à l'atmosphère (Deuxieme Memoire). *Ann. chim. phys.,* t. 69, pp. 353-367.

BURKE, R. T. A., and KOLBE, L. A.
1909. Soil survey of Rice County, Minnesota. U. S. Dept. Agr., Bur. of Soils, Field Operations, 1909, pp. 1269-1303, 1 fig., 1 map.

CAMERON, F. K., and BELL, J. M.
1905. The mineral constituents of the soil solution. U. S. Dept. Agr., Bur. of Soils, *Bull.* 30, 70 p

CAVENDISH, HENRY.
1784. Experiments on air. *Phil. Trans.,* v. 74, pp. 119-153.

CHARDET, PAR GASTON.
1914. La chimie du sol: l'évolution des acides aminés. *Rev. gen. chim.,* t. 17, pp. 137-144.

COHN, RUDOLF.
1896-97. Ueber eine quantitative Eiweisssplatung durch Salzsäure (I. Mittheilung). *Z. physiol. Chem.,* Bd. 22, pp. 153-175.

COHN, RUDOLF.
1898-99. Ueber eine quantitative Eiweisssplatung durch Salzsäure (II. Mittheilung). Auffindung eines Pyridinderivates. *Z. physiol. Chem.* Bd. 26, pp. 395-410.

DAKIN, H. D., and DUDLEY, H. W.
1914. Some limitations of the Kjeldahl method. *J. Biol. Chem.,* v. 17, pp. 275-280.

DAVY, SIR HUMPHRY.
1813. Elements of Agricultural Chemistry in a course of lectures before the Board of Agriculture, London. 4 to.

DECANDOLLE, AUGUSTIN PYRAMUS.
1833. Pflanzen-Physiologie, odor Darstellung der Lebenskräfte und Lebensverrichtungen der Gewachse. Eine Fortsetzung der Pflanzen-Organographie, und eine Einleitung zur Pflanzen-Geographie und okonomischen Botanik. Bd. I. 462 p. Translated from the French by Johannes Röper. J. G. Gotta, Stuttgart und Lübingen.

DETMER, W.
1871. Die natürlichen Humuskörper des Bodens und ihre landwirtschaftliche Bedeutung, Dissertation Leipzig. Also *Landw. Vers. Stat.*, 1871, Bd. 14, pp. 248-300. Abstracted in *Jahresber. d. Agr. Chem.*, 1870-72, pp. 68-74.

DOJARENKO, A.
1902. Der Stickstoff des Humus. *Ländw. Vers. Stat.*, Bd. 56, pp. 311-320.

EFFRONT, JEAN.
1914. Les Catalyseurs Biochimiques dans la Vie et dans l'Industrie. 772 p. H. Dunod et E. Pinat, Paris.

EGGERTZ, C. G.
1888. Studien und Untersuchungen über die Humuskörper der Acker- und Moorerde. *Meddelanden fran konigl. Landbruks-Akademiens Experimentalfält*, Nr. 3, Stockholm, pp. 1-66. Abstracted in *Centr. f. Agr. Chem.*, 1889, Bd. 18, pp. 75-80.

EHRLICH, FELIX.
1911. Über die Vergärung des Tyrosins zu p-oxyphenyl-äthylalkohol (Tyrosol). *Ber.*, Bd. 44, pp. 139-146.

EULER, HANS.
1912. General chemistry of the enzymes. Trans. by T. H. Pope, 323 p. John Wiley and Sons, New York.

FISCHER, EMIL.
1882. Ueber Caffeïn, Theobromin, Xanthin und Guanin. *Ann.*, Bd. 215, pp. 253-320.

FISCHER, EMIL.
1901. Ueber die Entstehung von *a*-Pyrrolidin-carbonsäure und Phenylalanin bei der Hydrolyse des Eieralbumins. *Z. physiol. Chem.*, Bd. 33, pp. 412-416.

FISCHER, EMIL.
1901. Ueber die Hydrolyse des Caseins durch Salzsäure. *Z. physiol. Chem.*, Bd. 33, pp. 151-176.

FISCHER, EMIL.
1899-06. Untersuchungen über Aminosäuren, Polypeptide und Proteïne, 770 p. Berlin.

FORBES, E. B., and KEITH, M. HELEN.
1914. A review of the literature of phosphorus compounds in animal metabolism. Ohio Agr. Exp. Sta., *Tech. Series*, Bull. 5, 748 p.

FOSSE, M. R.
1912. Recherches sur l'urée. *Compt. rend.*, t. 155, pp. 851-852.

FRAPS, G. S., and HAMNER, N. C.
1910. Studies on the ammonia-soluble organic matter of the soil. Tex. Agr. Exp. Sta., *Bull.* 53, 49 p.

GORTNER, R. A.
1913. Studies on the chemistry of embryonic growth. I. Certain changes in the nitrogen ratios in developing trout eggs. *J. Am. Chem. Soc.*, v. 35, pp. 632-645.

GORTNER, R. A.
1914. Studies on the chemistry of embryonic growth. II. Comparative analyses of the eggs and newly hatched larvæ of the giant salamander (Cryptobranchus Alleghaniensis). *J. Am. Chem. Soc.*, v. 36, pp. 1556-1566.

GORTNER, R. A.
1916a. The organic matter of the soil: I. Some data on humus, humus carbon, and humus nitrogen. *Soil Science*, v. 2, pp. 395-441. 2 pl.

GORTNER, R. A.
1916b. The organic matter of the soil: II. A study of carbon and nitrogen in seventeen successive extracts; with some observations on the nature of the black pigment of the soil. *Soil Science*, v. 2, pp. 539-548.

GORTNER, R. A.
1916c. The origin of the humin formed by the acid hydrolysis of proteins. II. Hydrolysis in the presence of carbohydrates and of aldehydes. *J. Biol. Chem.*, v. 26, pp. 177-204.

GORTNER, R. A.
1917. The organic matter of the soil: III. On the production of humus from manures. *Soil Science,* v. 3, pp. 1-8.

GORTNER, R. A., and BLISH, M. J.
1915. On the origin of the humin formed by the acid hydrolysis of proteins. *J. Am. Chem. Soc.,* v. 37, pp. 1630-1636.

GORTNER, R. A., and HOLM, GEORGE E.
1917. On the origin of the humin formed by the acid hydrolysis of proteins III. Hydrolysis in the presence of aldehydes II. Hydrolysis in the presence of formaldehyde. *J. Am. Chem. Soc.,* v. 39, pp. 2477-2501.

GORTNER, R. A., and SHAW, WILLIAM M.
1917. The organic matter of the soil: IV. Some data on humus phosphoric acid. *Soil Science,* v. 3, pp. 99-111.

GRANDEAU, LOUIS.
1872. Recherches sur le rôle des matières organiques du sol dans les phénomènes de la nutrition des végétaux. *Compt. rend.,* t. 74, pp. 988-991.

GRANDEAU, LOUIS.
1877. Traité d'Analyse des Matières agricoles. 487 p., 46 fig. Paris.

GRINDLEY, H. S., and SLATER, M. E.
1915. The quantitative determination of the amino-acids of feeding stuffs by the Van Slyke method. II. *J. Am. Chem. Soc.,* v. 37, pp. 2762-2769.

GROUVEN, H.
1883. Einer Abhandlung über die chemische Constitution der Moore. *Fühlings Landw. Zeitung,* p. 391. Abstracted in *Jahresber. Fort. Agr. Chem.,* N. S. Bd. 6, pp. 19-20.

HART, E. B. and SURE, BARNETT.
1916. The influence of carbohydrates on the accuracy of Van Slyke method in the hydrolysis of casein. *J. Biol. Chem.,* v. 28, pp. 241-249.

HAUSMANN, W.
1899. Ueber die Vertheilung des Stickstoffs im Eiweissmolekül. *Z. physiol. Chem.,* Bd. 27, pp. 95-108.

HELMONT, JOHANN BAPTIST VAN.
1648. Ortus medicinal vel opera et opuscula omnia. Cited in Friedrich Czapek's "Biochemie der Pflanzen." Bd. I. Jena 1913.

HENRIQUES, V.
1909. Über quantitative Bestimmung der Aminosäuren in Harne. *Z. physiol. Chem.,* Bd. 60, pp. 1-9.

HENRIQUES, V., and SÖRENSEN, S. P. L.
1910. Über die quantitative Bestimmung der Aminosäuren, Polypeptide und der Hippursäure im Harne durch Formoltitration, II. *Z. physiol. Chem.,* Bd. 64, pp. 120-143.

HILGARD, EUGENE W.
1906. Soils, their Formation, Properties, Composition, and Relations to Climate and Plant Growth in the Humid and Arid Regions. 593 p. The Macmillan Company, New York.

HLASIWETZ, H., and HABERMANN, J.
1871. Ueber die Proteïnstoffe. Erste Abhandlung. *Ann. der Chem. und Pharm.,* Bd. 159, pp. 304-333.

HLASIWETZ, H., and HABERMANN, J.
1873. Ueber die Proteïnstoffe. Zweite Abhandlung. *Ann. der Chem. und Pharm.,* Bd. 169, pp. 150-166.

HOPPE-SEYLER, F.
1909. Handbuch der physiologisch- und pathologisch-chemischen Analyse für Aerzte und Studierende. 8th edition, 854 p. August Hirschwald, Berlin.

JODIDI, S. L.
1909. Organic nitrogenous compounds in peat soils. Mich. Agr. Exp. Sta., *Tech. Bull.* 4, 28 p.

JODIDI, S. L.
1911. The chemical nature of the organic nitrogen in the soil. Iowa Agr. Exp. Sta., *Research Bull.* 1, 46 p., 1 fig.

JODIDI, S. L.
1914. Über den gegenwärtigen Stand der Bodenchemie mit besonderer Berücksichtigung der organischen Verbindungen. *Landw. Vers Stat.*, Bd. 85, pp. 359-391.

JONES, WALTER.
1914. Nucleic acids, their chemical properties and physiological conduct. 118 p. Longmans, Green and Company, New York.

KELLEY, W. P.
1914. The organic nitrogen of Hawaiian soils. I. The products of acid hydrolysis. *J. Am. Chem. Soc.*, v. 36, pp. 429-434.

KELLEY, W. P., and THOMPSON, ALICE R.
1914. The organic nitrogen of Hawaiian soils. Hawaii Agr. Exp. Sta, *Bull.* 33, 22 p.

KOSSEL, A.
1886. Weitere Beiträge zur Chemie des Zellkerns. *Z. physiol. Chem.*, Bd. 10, pp. 248-264.

KOSSEL, A., and STEUDEL, H.
1903. Weitere Untersuchungen über das Cytosin. *Z. physiol. Chem.*, Bd. 38, pp. 49-59.

LADD, E. F.
1898. Humates and soil fertility. *J. Am. Chem. Soc.*, v. 20, pp. 861-867.

LATHROP, E. C.
1912. Guanine from a heated soil. *J. Am. Chem. Soc.*, v. 34, pp. 1260-1263.

LATHROP, E. C.
1914. The nitrogen of processed fertilizers. U. S. Dept. Agr., *Bull.* 158, 24 p.

LATHROP, E. C.
1916. Protein decomposition in soils. *Soil Science*, v. 1, pp. 509-532.

LATHROP, E. C., and BROWN, B. E.
1911. Studies in organic soil nitrogen. *J. Ind. Eng. Chem.*, v. 3, pp. 657-660.

LAWES, J. B., and GILBERT, J. H.
1851. On agricultural chemistry, especially in relation to the mineral theory of Baron Liebig. *J. Roy. Agr. Soc.*, v. 12, pp. 1-40.

LIEBIG, JUSTUS VON.
1840. Die Organische Chemie in ihrer Anwendung auf Agricultur und Physiologie. Braunschweig. 8 vo.

LILIENFELD, LEON.
1892. Haematologische Untersuchungen. *Arch. Anat. Physiol.*, Bd. 7, pp. 115-154.

LOEW, O., and ASO, K.
1906-08. On changes of availability of nitrogen in soils. I. *Bull. Coll. Agr. Tokyo*, v. 7, pp. 443-448.

LOGES, G.
1886. Ueber Stickstoffhaltige organische Verbindungen im der Ackererde. *Landw. Vers. Stat.*, Bd. 32, pp. 201-202.

MANGUM, A. W., and SCHROEDER, F. C.
1906. Soil survey of the Crookston Area, Minnesota. U. S. Dept. Agr., Bur. of Soils, Field Operations, 1906, pp. 865-891, 1 fig., 1 map.

MATHEWS, ALBERT P.
1915. Physiological Chemistry. 1040 p. William Wood and Company, New York.

MULDER, GERRIT JAN.
1849. The Chemistry of Vegetable and Animal Physiology. Translated from the Dutch by P. F. H. Fromberg with an introduction and notes by James F. W. Johnson, Edinburgh, 1845-1849.

MULDER, GERRIT JAN.
1862. Die Chemie der Ackerkrume, Bd. II., p. 256. Nach der holländischen Original-Ausgabe deutsch bearbeitet und mit Erläuterungen versehen von Johannes Müller in Berlin. Berlin, 1861-1863, 3 vol. 8 vo.

OSBORNE, T. B., and HARRIS, I. F.
1903. Nitrogen and protein bodies. *J. Am. Chem. Soc.*, v. 25, pp. 323-353.

Osborne, T. B., and Jones, D. B.
1910. A consideration in the sources of loss in analyzing the products of protein hydrolysis. *Am. J. Physiol.*, v. 26, pp. 305-328.

Otori, J.
1904-05. Die Spaltung des Pseudomucins durch Starke siedende Säuren. II. Mitteilung. *Z. physiol. Chem*, Bd. 43, pp. 74-85.

Potter, R. S., and Snyder, R. S.
1914. The determination of ammonia in soils. Iowa Agr. Exp. Sta., *Research Bull.* 17, 19 p.

Potter, R. S., and Snyder, R. S.
1915a. Amino-acid nitrogen of soil and the chemical groups of amino acids in the hydrolyzed soil and their humic acids. *J. Am. Chem. Soc.*, v. 37, pp. 2219-2227.

Potter, R. S., and Snyder, R. S.
1915b. The amino acid nitrogen of soil. *J. Ind. Eng. Chem.*, v. 7, pp. 1049-1053.

Potter, R. S., and Snyder, R. S.
1916. Soluble nonprotein nitrogen in soil. *J. Agr. Res.*, v. 6, pp. 61-64.

Rimbach, C.
1900. Investigations on the determination and composition of humus and its nitrification. *J. Am. Chem. Soc.*, v. 22, pp. 695-703.

Ritthausen, H.
1877. Ueber hohe Gehalte an Stickstoff in Torf-Böden und humusreichen Mergeln und über die Bedeutung einiger Huminsäuresalze für die Ammoniakabsorption. *Fühlings Landw. Zeitung*, Bd. 26, pp. 161-167. Abstracted in *Centr. f. Agr. Chem.*, 1878, Bd. 7, pp. 95-101.

Robbins, William J.
1916. The cause of the disappearance of cumarin, vanillin, pyridine, and quinoline in the soil. *Science*, v. 44, pp. 894-895.

Robertson, R. A., Irvine, J. C., and Dobson, M. E.
1907. A contribution to the chemistry and physiological action of the humic acids. *Biochem. J.*, v. 2, pp. 458-480.

Robinson, C. S.
1911. Organic nitrogenous compounds in peat soils. II. Mich. Agr. Exp. Sta., *Tech. Bull.*, 7, 22 p.

Russell, Edward J.
1912. Soil Conditions and Plant Growth. 168 p. Longmans, Green and Company, London.

Samuely, Franz.
1902. Über die aus Eiweifs hervorgehenden Melanine. *Beitr. chem. Physiol. u. Path.*, Bd. 2, pp. 355-388.

Saussure, N. T. de.
1804. Recherches chimiques sur la végétation. Paris, 8 vo.

Scheele, Carl W.
1777. Chemische Abhandlung von der Luft und dem Feuer. Nebst einem Vorbericht von Torbern Bergman. Upsala and Leipzig. pp. VI-155. 12 mo.

Schiff, Hugo.
1900. Über Methylenasparagine. *Ann.*, Bd. 310, pp. 25-44.

Schiff, Hugo.
1901. Trennung von Amin- und Säurefunktion in Lösungen von Eiweisskörpern. *Ann.*, Bd. 319, pp. 287-303.

Schiff, Hugo.
1902. Trennung von Amin- und Säurefunktion mittelst Formaldehyd. (Dritte Mittheilung.) *Ann.*, Bd. 325, pp. 348-354.

Schittenhelm, A., and Schröter, F.
1904. Über die Spaltung der Hefenucleinsäure durch Bakterien, IV. Mittheilung. *Z. physiol. Chem.*, Bd. 41, pp. 284-292.

Schreiner, O., and Lathrop, E. C.
1911. Examination of soils for organic constituents, especially dihydroxystearic acid. U. S. Dept. Agr., Bur. of Soils, *Bull.* 80, 33 p., 2 pl.

Schreiner, O., and Lathrop, E. C.
1912. The chemistry of steam-heated soils. U. S. Dept. Agr., Bur. of Soils, *Bull.* 89, 37 p.

SCHREINER, O., and SHOREY, E. C.
1908a. The isolation of dihydroxystearic acid from soils. *Am. Chem. Soc.*, v. 30, pp. 1599-1607.

SCHREINER, O., and SHOREY, E. C.
1908b. The isolation of picoline carboxylic acid from soils and its relation to soil fertility. *J. Am. Chem. Soc.*, v. 30, pp. 1295-1307.

SCHREINER, O., and SHOREY, E. C.
1909. The presence of a cholesterol substance in soils; agrosterol. *J. Am. Chem. Soc.*, v. 31, pp. 116-118.

SCHREINER, O., and SHOREY, E. C.
1909a. The isolation of harmful organic substances from soils. U. S. Dept. Agr., Bur. of Soils, *Bull.* 53, 53 p., 4 pl.

SCHREINER, O., and SHOREY, E. C.
1910a. Chemical nature of soil organic matter. U. S. Dept. Agr., Bur. of Soils, *Bull.* 74, 41 p., 1 pl.

SCHREINER, O., and SHOREY, E. C.
1910b. Some acid constituents of soil humus. *J. Am. Chem. Soc.*, v. 32, pp. 1674-1680.

SCHREINER, O., and SHOREY, E. C.
1910c. Pyrimidine derivatives and purine bases in soils. *J. Biol. Chem.*, v. 8, pp. 385-393.

SCHREINER, O., and SHOREY, E. C.
1910d. The presence of arginine and histidine in soils. *J. Biol. Chem.*, v. 8, pp. 381-384.

SCHREINER, O., and SHOREY, E. C.
1911a. Paraffin hydrocarbons in soils. *J. Am. Chem. Soc.*, v. 33, pp. 81-83.

SCHREINER, O., and SHOREY, E. C.
1911b. Cholesterol bodies in soils: phytosterol. *J. Biol. Chem.*, v. 9, pp. 9-11.

SCHREINER, O., SHOREY, E. C., SULLIVAN, M. X., and SKINNER, J. J.
1911. A beneficial organic constituent of soils: creatinine. U. S. Dept. Agr., Bur. of Soils, *Bull.* 83, 44 p., 3 pl.

SESTINI, FAUSTO.
1899. Der die Humussäure im Erdreich und Torfe begleitende Stickstoffgehalt. *Landw. Vers. Stat.*, Bd. 51, pp. 153-158.

SHMOOK, ALEXANDER.
1914. "Some data pertaining to the forms of nitrogen in soils." (In Russian.) *Russ. J. Exp. Landwirtschaft*, Bd. 15, pp. 139-153.

SHOREY, E. C.
1905. Report of the chemist. *Rept. Agr. Invest. in Hawaii*, pp. 25-38.

SHOREY, E. C.
1906. Organic nitrogen in Hawaiian soils. *Rept. of Hawaii Agr. Exp. Sta.*, pp. 37-59.

SHOREY, E. C.
1911. The isolation of creatinine from soils. *Science*, v. 33, pp. 340.

SHOREY, E. C.
1911a. Nucleic acids in soils. *Biochem. Bull.*, v. 1, p. 104.

SHOREY, E. C.
1912. Nucleic acids in soils. *Science*, v. 35, p. 390.

SHOREY, E. C.
1913. Some organic soil constituents. U. S. Dept. Agr., Bur. of Soils, *Bull.* 88, 41 p., 1 pl.

SHOREY, E. C.
1914. The presence of some benzene derivatives in soils. *J. Agr. Res.*, v. 1, pp. 357-363.

SHOREY, E. C., and LATHROP, E. C.
1910. Pentosans in soils. *J. Am. Chem. Soc.*, v. 32, pp. 1680-1683.

SHOREY, E. C., and WALTERS, E. H.
1914. A nitrogenous soil constituent, tetracarbonimid. *J. Agr. Res.*, v. 3, pp. 175-178.

SIVERS, M. VON.
1880. Ueber der Stickstoffgehalt des Torfbodens. *Landw. Vers. Stat.*, Bd. 24, pp. 183-210.

SMITH, W. G., and KIRK, N. M.
1914. Soil survey of Ramsey County, Minnesota. U. S. Dept. Agr., Bur. of Soils, Field Operations 1914, Separate, 37 p., 2 fig., 1 map.

SNYDER, HARRY.
1895. Humus in its relation to soil fertility. Yearbook U. S. Dept. Agr., pp. 131-142.

SNYDER, HARRY.
1897. The composition of humus. *J. Am. Chem. Soc.*, v. 19, pp. 738-744.

SNYDER, HARRY.
1901. Humus and soil fertility. *Proc. Soc. Prom. Agr. Sci.*, Separate, 4 p.

SNYDER, HARRY.
1908. Soils and Fertilizers. Third edition, 350 p. The Macmillan Company, New York.

SÖRENSEN, S. P. L.
1907-08. Enzymstudien. *Biochem. Z.*, Bd. 7, pp. 45-101.

STOKLASA, J.
1911. Biochemischer Kreislauf des Phosphat-Ions im Boden. *Centr. Bakt. Parasitenk.*, II. Abt., Bd. 29, pp. 385-528.

SUZUKI, S.
1906-08a. On the formation of humus. *Bull. Coll. Agr. Tokyo*, v. 7, pp. 95-99.

SUZUKI, S.
1906-08b. Studies on humus formation. II. *Bull. Coll. Agr. Tokyo*, v. 7, pp. 419-423.

SUZUKI, S.
1906-08c. Studies on humus formation. III. *Bull. Coll. Agr. Tokyo*, v. 7, pp. 513-529.

THAER, ALBRECHT D.
1809-10. Grundsätze der rationellen Landwirthschaft. Realschulbuchhandlung, Berlin. 4 vol.

TRUSOV, A. G.
1915. "The humification of compounds entering the composition of plant organisms. III. The possibilities of humification of various organic compounds under the influence of natural reagents." (In Russian.) *Selsk. Khos. i. Liesov.*, v. 246, pp. 233-245.

VAN SLYKE, D. D.
1910. Eine Methode zur quantitativen Bestimmung der aliphatischen Aminogruppen; einige Anwendungen derselben in der Chemie der Proteine, des Harns und der Enzyme. *Ber.*, Bd. 43, pp. 3170-3181.

VAN SLYKE, D. D.
1911. The analysis of proteins by determination of the chemical groups characteristic of the different amino acids. *J. Biol. Chem.*, v. 10, pp. 15-55.

VAN SLYKE, D. D.
1912. The quantitative determination of aliphatic amino groups. II. *J. Biol Chem.*, v. 12, pp. 275-284.

VAN SLYKE, D. D.
1915. Improvements in the method for analysis of proteins by determination of the chemical groups characteristic of the different amino-acids. *J. Biol. Chem.*, v. 22, pp. 281-285.

VAUQUELIN.
1797. Sur une maladie des Arbres qui attaque spécialement l'Orme, et qui est analogue à un ulcère. *Ann. chim.*, t. 21, pp. 39-47.

WALTERS, E. H.
1915. The presence of proteoses and peptones in soils. *J. Ind. Eng. Chem.*, v. 7, pp. 860-863.

WALTERS, E. H., and WISE, L. E.
1916. *a*-Crotonic acid, a soil constituent. *J. Agr. Res.*, v. 6, pp. 1043-1046.

WARINGTON, R.
1887. Some recent researches on the nature of the nitrogenous organic matter of soils. *Chem. News*, v. 55, pp. 27-28.

WEIR, W.
1915. The effect of removing the soluble humus from a soil on its productiveness. *J. Agr. Sci.*, v. 7, pp. 246-253.

WISE, L. E., and WALTERS, E. H.
1917. Isolation of cyanuric acid from soil. *J. Agr. Res.*, v. 10, pp. 85-91.

WÖHLER, F.
1828. Ueber künstliche Bildung des Harnstoffs. *Poggendorff's Ann. Phys. u. Chem.*, Bd. 12, pp. 253-256.

BIOGRAPHICAL.

Clarence Austin Morrow was born near Morrow, Warren County, Ohio. He graduated from the Hillsboro, Ohio, high school in June, 1901. He entered the Ohio Wesleyan University in the fall of 1902, and received the degree of Bachelor of Science in June, 1906. During 1906-08 he held the position of Assistant in Chemistry at Oberlin College. He received the degree of Master of Arts from the same institution in June, 1909. In 1909-10 he was acting head of the Departments of Chemistry and Physics at Doane College. Having been appointed the John Harrison Scholar in Chemistry at the University of Pennsylvania he entered that institution in the fall of 1910 and continued his graduate work. In the spring of 1911 he was appointed John Harrison Fellow in Chemistry, but later resigned this to take the position of Professor of Chemistry at Nebraska Wesleyan University, which position he has held to date (January, 1917). During 1914-15 he was granted leave of absence for study in the Division of Soils, University of Minnesota, where he held the position of Assistant in Soil Chemistry. Here he studied for the degree of Doctor of Philosophy.

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